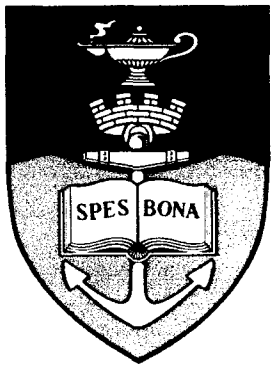

Molecular Characterization of the *Arabidopsis thaliana* - *Botrytis cinerea* Interaction

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B.Sc. (Agric.) *Hons*, M.Sc.(Crop Science)

Thesis Presented for the Degree of
DOCTOR OF PHILOSOPHY
in the Department of Molecular and Cellular Biology
UNIVERSITY OF CAPE TOWN

February 2008

Dedicated to my mother, Ms. Harriet Kigongo Nakibuuka; wife, Annet Abenakyo Mulema; children, sisters, and friends.

Declaration

I hereby declare that the work presented in this thesis is a result of my own original research. I also affirm that this work has not been presented in this, or any other University for examination, or for any other purposes.

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February 2008

Acknowledgements

I am very grateful to the Rockefeller Foundation for having funded the study through a grant offered to USHEPiA at the University of Cape Town. I also acknowledge the international travel grant offered to me by the University of Cape Town which enabled me to spend valuable academic time at the University of Warwick in the United Kingdom.

I profoundly acknowledge all academics who have been very influential in my studies especially Dr. Katherine Denby, my principal PhD supervisor. Her guidance, support and encouragement have been the recipe for my success. I also thank Professor Adipala Ekwamu whose tireless efforts to see that I study can not go unrecognized and Dr. Patrick Okori for his invaluable assistance.

I would like in a special way to thank my entire family; my mother, Ms. Harriet Kigongo Nakibuuka; wife, Annet Abenakyo Mulema; son, Gabriel Kusasira Mulema, and sisters; Dr. Namulema Teddy, Nangendo Clare, Nakafumbe Oliver and Nabaggala Goreth. I do acknowledge all the sacrifices you endured to see that I complete my doctoral degree.

I express my sincere gratitude to the USHEPiA Office: Ms Nan Warner, Ms Zubaida Hattas, and Ms. Masego Mogodu; All Africa House management; the Denby Lab: Dr. Shane Murray, Dr. Rob Ingle, Dr. Stuart Meier, Dr. Tichaona Mangwende, Dr. Lindsay Petersen, Maryke Carstens, Linda Wei, Nicki Adams, and Lara Donaldson; CAPAR: Tsion Abraha, Sally Ann Walford and Rachel Barrow and the Proteomics facility at the University of Warwick: Prof. Jim Scrivens, Ms. Susan Slade, Dr. Konstantinos Thalassinos, Nisha Patel, Vibhuti Patel, Richard Holland, Georgios Efstathiou, Charlie Scarff and Gill Hilton for all your priceless assistance during the project. Lastly, to all my friends especially USHEPiA PhD fellows, it was an amazing experience to have met you all.

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Abbreviations

ABA	Abscisic acid.
ACC	1-aminocyclopropane-1-caboxylase.
α	Alpha.
AOS	Active oxygen species.
AVG	Aminoethoxyvinylglycine.
avr	avirulence.
BABA	β-aminobutyric acid.
BAC1	*Botrytis cinerea* adenylate cyclase 1.
Bcaba1	*Botrytis cinerea* abscisic acid 1.
Bcaba2	*Botrytis cinerea* abscisic acid 2.
Bcaba3	*Botrytis cinerea* abscisic acid 3.
Bcaba4	*Botrytis cinerea* abscisic acid 4.
Bcap1	*Botrytis cinerea* aspartic proteinase 1.
Bcap2	*Botrytis cinerea* aspartic proteinase 2.
Bcap3	*Botrytis cinerea* aspartic proteinase 3.
Bcap4	*Botrytis cinerea* aspartic proteinase 4.
Bcap5	*Botrytis cinerea* aspartic proteinase 5.
Bcbot1	*Botrytis cinerea* botrydial 1.
Bccat1	*Botrytis cinerea* catalase 1.
Bccat2	*Botrytis cinerea* catalase 2.

BCG1	*Botrytis cinerea* Gα1.
BCG2	*Botrytis cinerea* Gα2.
BCG3	*Botrytis cinerea* Gα3.
Bcgod1	*Botrytis cinerea* glucose oxidase 1.
Bcgst1	*Botrytis cinerea* glutathione S-transferase 1.
Bchsp30	*Botrytis cinerea* heat shock protein 30.
BcoahA	*Botrytis cinerea* oxaloacetate acetylhydrolase A.
Bcpg1	*Botrytis cinerea* endopolygalacturonase 1.
Bcpg2	*Botrytis cinerea* endopolygalacturonase 2.
Bcpg3	*Botrytis cinerea* endopolygalacturonase 3.
Bcpg4	*Botrytis cinerea* endopolygalacturonase 4.
Bcpg5	*Botrytis cinerea* endopolygalacturonase 5.
Bcpg6	*Botrytis cinerea* endopolygalacturonase 6.
BcPLS1	*Botrytis cinerea* tetraspanin 1.
Bcpme1	*Botrytis cinerea* pectin methylesterase 1.
Bcpme2	*Botrytis cinerea* pectin methylesterase 2.
Bcsak1	*Botrytis cinerea* stress-activated kinase 1.
Bcsod1	*Botrytis cinerea* superoxide dismutase 1.
Bcspl1	*Botrytis cinerea* snod-prot-like 1.
β	Beta.
BKI	BestKeeper index.
BMP1	*Botrytis cinerea* MAP Kinase 1.
Bos1	*Botryotinia* osmotic sensitivity 1.
BOS1	*Botrytis*-susceptible 1.
BOS2	*Botrytis*-susceptible 2.
BOS3	*Botrytis*-susceptible 3.

BOS4	*Botrytis*-susceptible 4.
BSA	Bovine serum albumin.
BTH	Benzothiadiazole.
BTP1	*Botrytis cinerea* transmembrane protein 1.
cAMP	Cyclic adenosine monophosphate.
cDNA	Complementary DNA.
CGA1	*Cochliobolus heterostrophus* Gα1.
CHAPS	3-((3-Cholamidopropyl)dimethylammonio)-1-propanesulfonic acid.
CHIB	Chitinase B.
CHK1	*Cochliobolus heterostrophus* MAP Kinase 1.
cir3	Constitutively induced resistance 3.
ClPLS1	*Colletotrichum lindemuthianum tetraspanin 1*.
CMK1	*Colletotrichum lagenarium* MAP Kinase 1.
coi1	Coronatine insensitive 1.
CPG1	*Cryphonectria parasitica* Gα1.
CpMK1	*Cryphonectria parasitica* MAP Kinase 1.
CTG1	*Colletotrichum trifolii* Gα1.
CWDEs	Cell wall degrading enzymes.
Cy3	Cyanine 3.
Cy5	Cyanine 5.
DAB	3,3-Diaminobenzidine.
def1	Defenseless 1.
DEPC	Diethylpyrocarbonate.
DMSO	Dimethyl sulfoxide.
dNTP	Deoxyribonucleotide triphosphate.
dpi	Days postinoculation.

DTT	Dithiothreitol.
EC	Enzyme commission number.
EDS1	Enhanced disease susceptibility 1.
EDS4	Enhanced disease susceptibility 4.
EDS5	Enhanced disease susceptibility 5.
EDTA	Ethylene diamine tetra-acetate.
EIN2	Ethylene insensitive 2.
ERF1	Ethylene response factor 1.
ESA1	Enhanced susceptibility to *Alternaria brassicicola*.
EST	Expressed sequence tag.
ET	Ethylene.
etr1	Ethylene triple response 1.
FDR	False discovery rate.
FsMAPK	*Fusarium solani* f.sp. *pisi* MAP Kinase.
FWER	Family-wise error rate.
γ	Gamma.
GANB	Gα -protein from *Aspergillus nidulans* B.
GASC	Gα-subunitC.
GPA3	G protein α3.
GPCRs	G protein-coupled receptors.
GST	Glutathione S-transferase.
GUS	β-glucuronidase.
H_2O_2	Hydrogen peroxide.
HEL	Hevein-like protein.
HPCF	High performance chromatofocusing.
hpi	Hours postinoculation.

HPRP	High performance reverse-phase.
HR	Hypersensitive response.
ICAT	Isotope-coded affinity tagging.
ICS	Isochorismate synthase.
INA	2,6-Dichloroisonicotinic acid.
iop1	Induced over-expressor of *PDF1.2*.
iTRAQ	Isobaric tags for relative and absolute quantification.
JA	Jasmonic acid.
jar1	Jasmonic acid resistant 1.
$K_4Fe(CN)_6$	Potassium ferrocyanide.
KCl	Potassium chloride.
KMBA	α-keto-γ-methylbutyric acid.
LC	Liquid chromatography.
LiCl	Lithium chloride.
LOESS	Locally weighted scatterplot smoothing.
LRR	Leucine rich repeat.
MAGA	*Magnaporthe grisea* GαA.
MAGB	*Magnaporthe grisea* GαB.
MAMPs	Microbe-associated molecular patterns.
MapIII	MAP Kinase III.
MAPK	Mitogen-activated protein kinase.
MAPKK	Mitogen-activated protein kinase kinase.
MAPKKK	Mitogen-activated protein kinase kinase kinase.
MeJA	Methyl jasmonate.
$MgCl_2$	Magnesium chloride.
MgPLS1	*Magnaporthe grisea tetraspanin 1*.

MIMPs	Microbe-induced molecular patterns.
MPK4	MAP KINASE 4.
NaCl	Sodium chloride.
$NaHCO_3$	Sodium bicarbornate.
NahG	Salicylate hydroxylase.
NaOH	Sodium hydroxide.
Na_3PO_4	Sodium phosphate.
NH_4HCO_3	Ammonium bicarbonate.
NO	Nitric oxide.
npr1	Non-expressor of PR1.
OGAs	Oligogalacturonides.
Os-2	Osmotic sensitivity 2.
Osm1	Osmosensensive 1.
PAD2	Phytoalexin deficient 2.
PAD4	Phytoalexin deficient 4.
PAGE	Polyacrylamide gel electrophoresis.
PAL	Phenylalanine ammonia lyase.
PAMPs	Pathogen-associated molecular patterns.
PCR	Polymerase chain reaction.
PDF1.2	Plant defensin 1.2.
PGA	Polygalacturonic acid.
PGIPs	Polygalacturonase-inhibiting proteins.
PKA	Protein kinase A.
PMK1	*Magnaporthe grisea* MAP Kinase 1.
PMSF	Phenylmethanesulfonyl fluoride.
QTL	Quantitative trait loci.

RILs	Recombinant inbred lines.
RNA	Ribonucleic acid.
SA	Salicylic acid.
SAM	S-adenosyl methionine.
SAR	Systemic acquired resistance.
SB3-10	N-decyl-N,N-dimethyl-3-ammonio-1-propane sulfonate.
SDS	Sodium dodecyl sulfate.
SGT1	Suppressor of the G2 allele of *SKP1*.
SID2	Salicylic acid induction deficient 2.
SILAC	Stable isotope labelling in cell culture.
SOD	Superoxide dismutase.
SSC	Saline sodium citrate.
Taq	*Thermus aquaticus*.
TCEP	Tris (2-carboxyethyl)phosphine hydrochloride.
TEMED	N,N,N',N'-Tetramethylethylenediamine.
TFA	Trifluoroacetic acid.
THI2.1	Thionin 2.1.
TIFF	Tagged image file format.
TLC	Thin layer chromatography.
ubc3	*Ustilago* bypass of cyclase 3.
UV	Ultraviolet.
X-Gluc	5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid.

Molecular Characterization of the *Arabidopsis thaliana* - *Botrytis cinerea* Interaction

Mulema Joseph Mary K.

Thesis presented for the degree of Doctor of Philosophy, February 2008

Plants activate an array of defence responses following recognition of pathogenic organisms. This study attempted to characterize at a transcriptional level, the defence responses of *Arabidopsis thaliana* after infection by *Botrytis cinerea* using microarrays. The first microarray experiment focused on profiling *Arabidopsis* genes induced by *B. cinerea* over time (temporal) while the second investigated spatial expression of *Arabidopsis* genes from the point of inoculation. A number of genes were up- and down-regulated specifically at 12 hrs, others at 24 hrs while others were up- and down-regulated at both time points. Similarly, some genes were specifically induced very close to the lesion while others in more distal tissue. Microarray results were confirmed for a subset of genes from the temporal experiment using quantitative PCR and *Arabidopsis* transgenic lines carrying promoter:reporter gene fusions. In both cases, a trend in gene expression similar to that in microarrays was observed. Gene knockouts were obtained for some of the up-regulated genes, however, a mild phenotype was observed in only one mutant, an indication of redundancy or that key genes have not been identified. Clustering of expression profiles indicated a large overlap of gene expression with those following infection with other pathogens or abiotic stress conditions. A proteomic expression study using two techniques, 2D SDS-PAGE and 2D liquid chromatography was also carried out to determine whether levels of proteins corresponding to up- and down-regulated genes also increased or reduced in abundance after infection of *A. thaliana* with *B. cinerea*. Most of the proteins whose levels changed in abundance were those involved in defence responses to *B. cinerea* and were induced in the microarray study. This study therefore highlighted a multitude of genes induced in *Arabidopsis* spatially and temporally following infection with *B. cinerea* providing insight into key processes of defence against this pathogen. Furthermore this study has identified a plethora of candidate genes for further investigation into whether they are essential in defence against *B. cinerea* and restricting the degree of susceptibility of the host.

Chapter 1

Introduction

1.1 *Botrytis cinerea*

Botrytis cinerea Per. Fr. (anamorph) is one of the most important plant pathogens affecting the horticultural industry anywhere in the world (Michailides and Elmer, 2000; Omunyin and Mutiga, 2004). It is the causal agent of grey mold, a disease that has been reported on more than 200 plant species including many economically important crops (Jarvis, 1977). It mostly affects dicotyledons and corroliferous monocotyledons because they contain high contents of its preferred substrate, pectin (Carpita and Gibeaut, 1993). Hosts of *B. cinerea* include grapes, strawberries, raspberries, tomatoes, cucumber, roses, gerbera, onions and many other field crops (Grigaliunaite, 2001; Baraldi *et al.*, 2002; Staats *et al.*, 2005). *B. cinerea* affects the fruits and berries on grapes, strawberries, raspberries and tomatoes, bulbs on onions and flowers on roses and gerbella. Besides having a wide host range, *B. cinerea* can infect any part of the plant at any stage of growth although it is more destructive on mature or senescent tissues (Williamson *et al.*, 2007). The pathogen is responsible for pre-harvest as well as massive post-harvest damage reported in many crops especially fruits and vegetables (Williamson *et al.*, 2007).

Post-harvest damage not only decimates the overall crop yield but also results in reduced quality of most horticultural products such as ghost spot in tomato (Williamson *et al.*, 2007). Post-harvest losses attributable to *B. cinerea* may go beyond 50 percent however, with appropriate control measures and management practices, these may be reduced to between 5 and 10 percent (Margosan *et al.*, 1997; Droby and Lichter, 2004). *B. cinerea* has also been reported to cause allergic reactions in humans especially those continuously exposed to it in wine industries. They contract a condition referred to as winegrower's lung, a form of *hypersensitivity pneumonitis* (Zuskin *et al.*, 1997; Scott, 2001). *Hypersensitivity pneumonitis* also known as *extrinsic allergic alveolitis*, is the inflammation of the peripheral airways and surrounding interstitial tissue caused by inhalation of allergens such as small airborne particles (Ashok *et al.*, 2001). Although *B. cinerea* is a menace to the horticultural industry, the fungus is valued by the wine industry where it is commonly referred to as "noble rot". Infection by *B. cinerea* results in evaporation of water from the grapes causing them to shrivel. This leaves shriveled grapes with a high percentage of sugars and acids which are ideal for concentrated wines.

Because of its importance and wide spread distribution, *B. cinerea* has been widely studied. Information in areas of parasitism, physiology, biochemistry, epidemiology and control is readily available and with the sequencing of the genome, opportunities for functional genomics have been created. Studying gene function is also possible because this pathogen can be transformed with success using a number of methods such as gene disruption (Prins *et al.*, 2000b), gene replacement (Kars *et al.*, 2005a), and T-DNA insertional mutagenesis (Rolland *et al.*, 2003). The pathogen is very easy to culture in the laboratory and releases an abundant mass of disease propagules. All put together, *B. cinerea* provides a good model for understanding pathogenesis in necrotrophic fungi.

1.2 Biology

Botrytis cinerea is a filamentous, heterothallic fungus (Giraud *et al.*, 1997). The fungus is estimated to have a genome size of 38 Mb containing between 10,000-12,000 genes spread over 10 chromosomes (http://www.broad.mit.edu). It reproduces predominantly by asexual means through multinucleate macroconidia (conidia) which are also the main disease propagules (Jarvis, 1977). These are produced continuously throughout the growing season and can be dispersed over long distances with the aid of wind, air currents, rain splash and insects (Coley-Smith, 1980; Jarvis, 1980a; Kerssies, 1993). Conidia are unicellular and ellipsoid and measure on average 6-18 x 3.5-12 μm (Grigaliunaite, 2001). When single-celled, they are hyaline or pale brown and grey brown when in mass (Grigaliunaite, 2001). They are produced on apically branching conidiophores resulting from the mycelia. Conidiophores measure 2 μm or longer, mostly 16-30 μm thick, often septate, smooth-walled, clear brown and bearing numerous conidia (Jarvis, 1977).

The fungus also produces numerous uninucleate microconidia. These act as male spermatia and are thus responsible for initiating the sexual reproductive cycle by mating with sclerotia (Jarvis, 1980b). They are spherical in shape and about 1 μm in diameter. They may form on the tips of phialides on germ tubes from hyphae elongated from macroconidia or from old hyphae (Fukumori *et al.*, 2004). The products of sexual reproduction are apothecia which contain asci. Asci

are cylindrical, hyaline and evanescent at maturity (Fukumori *et al.*, 2004). They measure 136-184 x 9.7-17.5 μm and contain eight ascospores. Ascospores are hyaline, one-celled, ellipsoid and measure 4.5-5.5 x 9.7-14.5 μm (Fukumori *et al.*, 2004). In addition to conidia, mycelia and sclerotia, ascospores can also initiate epidemics over a wide range of temperatures (3-27^{o}C) (Jarvis, 1980a). The sexual, perfect or telomorphic state of *B. cinerea* is referred to as *Botryotinia fuckeliana* (de Bary Whetzel) (Jarvis, 1977).

B. cinerea survives unfavourable conditions over long periods as sclerotia which develop in dead plant tissue (Coley-Smith, 1980). Sclerotia are protected from dessication, UV radiation and microbial attack by a melanized rind and β-glucan encasing (Backhouse and Willets, 1984). It may also survive as mycelium which remain in infected decaying plant debris (Coley-Smith, 1980). Mycelia are also the principal means by which *B. cinerea* survives between seasons. *B. cinerea* is a known necrotroph and like all necrotrophs, it relies primarily on its ability to kill plant cells so as to establish infection and therefore start a parasitic life cycle (Zheng *et al.*, 2000; Kars *et al.*, 2005a). Besides leading a parasitic life, it can also survive as a saprophyte as well as a secondary invader (i.e. attacking plants that have been infected by other pathogens) (Collado *et al.*, 2000).

1.3 Taxonomy

Botrytis cinerea is classified under the division, *Deuteromycota*; class, *Leotiomycetes*; order, *Moniliales*; family, *Moniliaceae* and genus, *Botrytis* while the sexual *B. fuckeliana* is classified under the division, *Ascomycota*; class, *Discomycetes*; order, *Helotiales*; family, *Sclerotiniaceae* and genus *Botryotinia*. The genus *Botrytis* contains many other species that are important crop pathogens, however, unlike *B. cinerea* they are considered to be specialists with a narrow host range (Staats *et al.*, 2005). They infect only one or a few closely related species within the same plant genus (Staats *et al.*, 2005). Specialized members occur on corroliferous monocotyledons and on members of the four eudicot families *Fabaceae*, *Ranunculaceae*, *Geraniaceae* and *Paeoniaceae*. Examples include *B. anthophila* which affects red clover (*Trifolium pratense*) (Grigaliunaite, 2001); *B. elliptica* of lillies (*Lilium* spp) (Grigaliu-

naite, 2001); *B. gladiolorum* of gladiolus (*Gladiolus communis*) (Grigaliunaite, 2001); *B. tulipae*, of tulips (*Tulipa gesneriana*) (Grigaliunaite, 2001) and *B. paeoniae* on paeony (*Paeonia* spp) (Grigaliunaite, 2001).

Some specialists may infect several members of the same plant family or genera. An example is *B. fabae* which can infect species of the genera *Vicia*, *Lens*, *Pisum* and *Phaseolus* all of which belong to *Fabaceae* (Hashim *et al.*, 1997) and *B. allii*. *B. byssoidea* and *B. squamosa* all of which attack members of the *Allium* spp such as chives, leek, garlic and onions (Chilvers *et al.*, 2004; Grigaliunaite, 2001). Host specificity is dependent on a number of factors which include production of host-specific toxic factors such as those produced by *B. fabae* (Staats *et al.*, 2005) and *B. elliptica* (van Baarlen *et al.*, 2004) and the ability to overcome specific phytoalexins produced by hosts, for instance broad beans produce a group of furanoacetylenic phytoalexins which are toxic to all *Botrytis* species except *B. fabae* and tulip bulbs produce tulipalins which can only be overcome by *B. tulipae* (van Baarlen *et al.*, 2007).

1.4 Genetic diversity

Botrytis cinerea has undergone more population genetic studies than any other *Botrytis* species. All these studies have concurred that the fungus exhibits a great somatic, metabolic and genetic diversity. This is manifested in characteristics such as mycelial growth rate, incidence of sporulation structures and sclerotia, pathogenicity and resistance to fungicides especially benzimidazoles and dicarboximides (Faretra and Pollastro, 1991; van der Vlugt-Bergmans *et al.*, 1993; Diolez *et al.*, 1995; Kerssies *et al.*, 1997; Alfonso *et al.*, 2000; Baraldi *et al.*, 2002). Many factors such as aneuploidy, heterokaryosis, parasexuality and mutation have been put forward to explain this diversity (van Kan *et al.*, 1993; Vallejo *et al.*, 1996; Giraud *et al.*, 1997; Baraldi *et al.*, 2002). Although *B. cinerea* can form sexual structures (apothecia) with any strain under laboratory conditions, these structures are seldom observed in the field (Faretra *et al.*, 1988; Fukumori *et al.*, 2004). Therefore, genetic recombination through sexual reproduction on its own may not offer a definitive explanation.

However, studies carried out by Giraud *et al.* (1997), in different countries have shown that mating type alleles; *MAT1-1* and *MAT1-2* are distributed in equal frequencies in *Botrytis* populations sampled from the same area (Beever and Parkes, 1993; Faretra and Pollastro, 1993). Giraud *et al.* (1997) attributed this to the existence of sexual reproduction which only has the ability to exert the selection pressure required to keep allele frequencies at unity and prevent genetic drift. Genetic diversity due to genetic recombination was also supported by Alfonso *et al.* (2000). Recent studies by Staats *et al.* (2005), have shown that sexual reproduction could have been lost in *Botrytis* species as a result of negative selection brought about by a change in habits; an indication that it does not play a significant role.

Using restriction fragment length polymorphism (RLFP) markers, Giraud *et al.* (1997) carried out population studies on *B. cinerea* isolates in the Champagne region in France and concluded that they consisted of at least two sympatric species, *transposa* which was characterised by the presence of the transposable elements *Boty* and *Flipper*, and *vacuma* which had neither of these transposable elements. The *transposa* species were more clustered while *vacuma* isolates were more scattered suggesting heterogeneity. The transposable element *Boty* transposes by reverse transcription while *Flipper* transposes directly using the enzyme transposase (Diolez *et al.*, 1995; Levis *et al.*, 1997; Fávaro de Lima *et al.*, 2005). *Transposa* and *vacuma* were more frequent on asymptotic tissue and disease lesions respectively an indication that *vacuma* acts more as a saprophyte while *transposa* acts more as a parasite (Giraud *et al.*, 1999). Studies in California (Ma and Michailides, 2005) and Chile (Muñoz *et al.*, 2002) showed that *transposa* and *vacuma* were not restricted to the Champagne region in France.

Albertini *et al.* (2002) and Fournier *et al.* (2003) studied DNA polymorphism at two different nuclear genes, eburicol 14α-demethylase gene (*CYP51*) and Bc-*hch* respectively. The vegetative compatibility locus, Bc-*hch* is a homolog of Nc-*het-c* and Pa-*hch* of *Neurospora crassa* and *Podospora anserina* respectively. They also showed that *B. cinerea* isolates were clustered in two different genetically isolated subgroups. However, the structure within each subgroup differed from that proposed by Giraud *et al.* (1997). Group I consisted of isolates of the *vacuma* type that were resistant to the fungicide fenhexamid while group II consisted of isolates of either

the *transposa* or *vacuma* type and all were sensitive to fenhexamid.

Recently, Fournier *et al.* (2005) used the multiple-gene genealogies approach to demonstrate that *B. cinerea* is indeed a species complex comprising of two phylogenetic species. Group I consisted of *vacuma* while the other group consisted of *vacuma* and *transposa*. The two groups appeared to have been strongly isolated, exchanging no migrants for a long time, which could likely be due to a barrier to gene flow. This hypothesis put forward by Giraud *et al.* (1997) was confirmed by the existence of a high number of polymorphic sites fixed within each group. DNA polymorphism and vegetative compatibility tests revealed that genetic diversity was lower in group I than II. The two groups also exhibited differences in phenology, host range, size of asexual spores, and vegetative compatibility. All these results indicated that *B. cinerea* groups I and II could be different cryptic species isolated for a long time.

1.5 Disease symptoms

The most observed symptoms of diseases caused by *B. cinerea* include a grey to brown discolouration, water soaking and a fuzzy whitish grey to tan mold which grows on the surface of affected areas. However, symptoms do greatly vary depending on the host, plant part and stage in growth that is attacked (Jarvis, 1977). In seed-propagated plants such as ornamentals, vegetables, and forest trees, *B. cinerea* causes pre- and post-emergence damping-off (Hausbeck and Moorman, 1996). Pre-emergence damping-off occurs when the fungus infects developing radicals and kills seedlings while shoot tissues are still below ground. This could possibly be caused by seed-borne infections, a possibility demonstrated by Burgess *et al.* (1997) in Australia.

On leaves and flowers, blights are the most common symptom (Hausbeck and Moorman, 1996). Blights begin as small water-soaked spots which rapidly coalesce affecting large potions of the tissue. Infection of flowers may cause premature fading and dying of petals which then drop on healthy leaves initiating leaf blights. Leaf symptoms first appear as small, soft, yellowish or tan spots which later become whitish grey or tan. They may enlarge and coalesce to a point where they can de-

stroy the whole leaf. Stems may also be infected either through scars, wounds or leaf infections which progress into the stalk and eventually the stem (Palmucci and Grijalba, 2005). Stem lesions are dark, sunken and elongated with distinct margins. If lesions spread throughout the whole stem, it may result in death of the plant. In cold wet weather, the infected area of the stem may develop a greyish mold.

B. cinerea becomes a serious pathogen at the time the plant starts flowering onwards. This is because early-season infections are often limited by physical and chemical host defences which cause the pathogen to enter a quiescent or latent phase in the host tissue (Elmer and Reglinski, 2006). However, as the plant starts to age, a deterioration in many departments necessary to fight infection results which *B. cinerea* capitalizes on (Elad and Evensen, 1995). Infection of fruits usually occurs at flowering time, the fungus grows from the fading flower petals into the developing fruit causing blossom-end rot. This can be followed by complete fruit destruction. Infected fruits develop water-soaked, yellowish green or greyish brown irregular lesions. Although *B. cinerea* can start destroying the fruit as it develops as in the case of blossom-end rot and ghost spot, in some cases the fungus may remain in the developing fruit but only start the infection process at the beginning of ripening (Baraldi *et al.*, 2002; Keller *et al.*, 2003; Pezet *et al.*, 2003).

1.6 Disease cycle

When conditions that favour disease development such as cool (10-23^{o}C) moist damp weather occur, sclerotia germinate to produce an abundant mass of grey mycelium which in turn produce conidiophores containing numerous conidia. When conidia land on a susceptible host, they germinate and penetrate tissue either directly or through natural openings such as stomata, lenticels and hydathodes or through wounds like those caused by feeding insects (Jarvis, 1977; Holz *et al.*, 2004). After penetrating the epidermis and killing the underlying cells, *B. cinerea* establishes primary lesions with defined margins. These necrotic lesions can expand rapidly if plant defence systems are weakened, resulting in secondary lesions and extensive maceration of the plant tissue. Finally, the fungus sporulates on the damaged tissue to produce inoculum for the next infection cycle (Fig. 1.1).

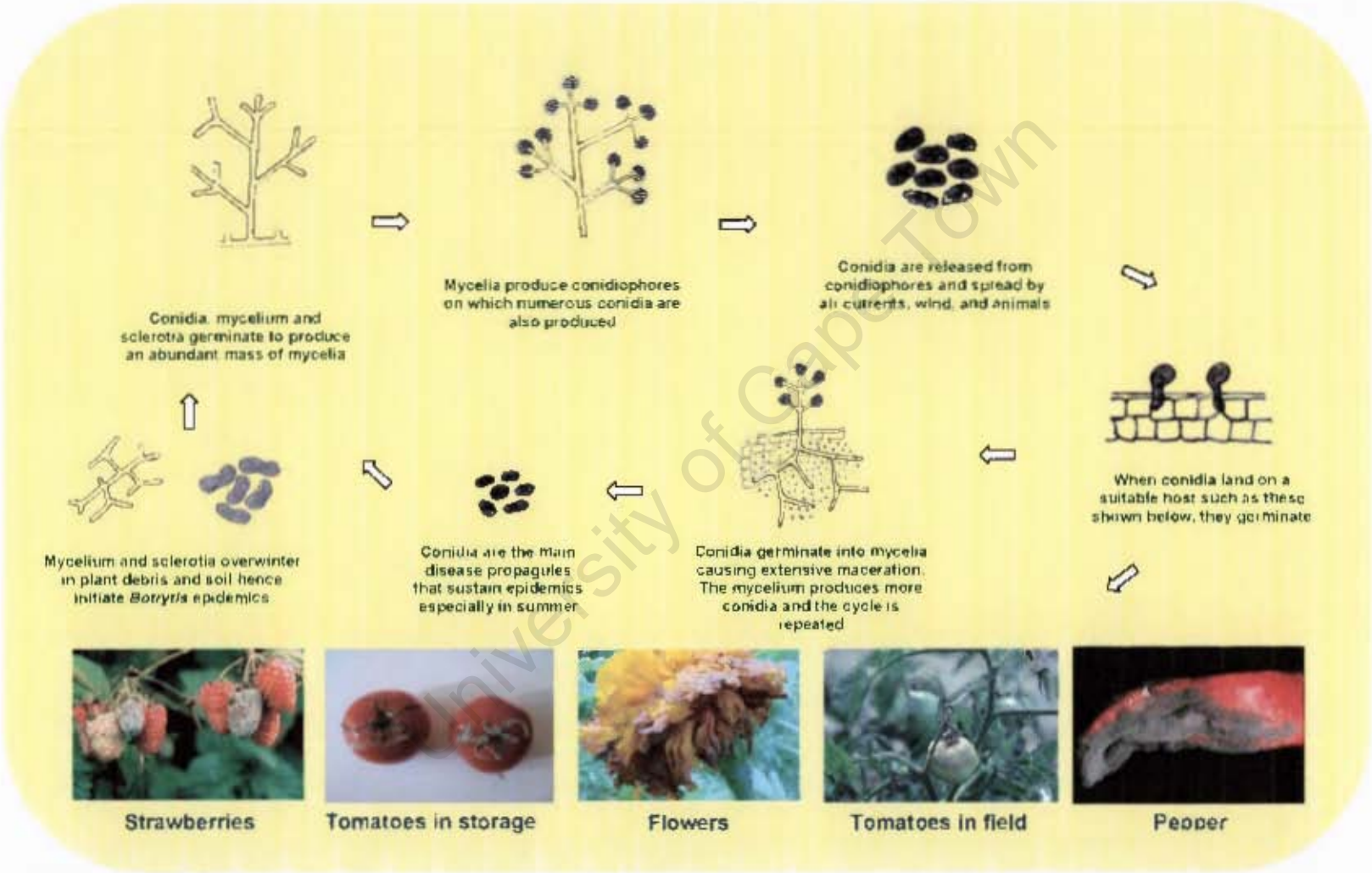


Figure 1.1: Life cycle of any *Botrytis* species: The image has been recreated based on Agrios (1997)

1.7 Pathogenesis

Epidemics of *B. cinerea* are initiated from mycelium or sclerotia but are sustained by conidia (Coley-Smith, 1980). When conidia land on a suitable host, they get attached to the substratum by means of an adhesive mucilaginous sheath (Jarvis, 1977). This attachment occurs in two distinct stages; immediate adhesion which occurs upon hydration and is characterised by relatively weak adhesive forces (Doss *et al.*, 1993) and delayed adhesion which occurs after viable conidia have been incubated for several hours under conditions that promote germination (Doss *et al.*, 1995). Following attachment, conidial germination is influenced by factors such as surface hardness, hydrophobicity, presence of nutrients and water. Germination of *B. cinerea* conidia on hydrophobic surfaces can occur in water while germination on hydrophillic surfaces requires nutrients (Doehlemann *et al.*, 2005, 2006). Nutrients necessary for germination include sugars (e.g. fructose, glucose and maltose) and amino acids. These nutrients are contained in pollen or plant exudates released by the host or may originate from external sources such as insect secretions (Elad, 1997). The fact that the process is influenced by surface hardness indicates that *B. cinerea* senses the chemical and physical qualities of its environment during germination (van Kan, 2006). These early processes of the infection process seem to be regulated by intracellular signalling cascades especially the mitogen activated protein kinase (MAPK), cyclic adenosine monophosphate (cAMP) and Ca^{2+}/calmodulin-dependent signalling cascades.

1.7.1 MAPK signalling

MAP kinase cascades are important in helping organisms perceive changes in their environment and responding to these changes by adjusting their intracellular activities. Organisms achieve this by controlling expression of genes involved for instance in stress response, cell division, differentiation, cell survival, and apoptosis as a reaction to diverse extracellular triggers (Treisman, 1996; Banuett, 1998; Gustin *et al.*, 1998). Components that make up this cascade include a MAP kinase kinase kinase (MAPKKK) which phosphorylates a MAP kinase kinase (MAPKK) which in turn phosphorylates a MAP kinase (MAPK). MAPK cascades relay the perception of an

external stimulus by a receptor into intracellular responses (Widmann *et al.*, 1999; Chang and Karin, 2001). MAP kinases are highly conserved in eukaryotes and have been identified in a variety of organisms from yeast to human (Xu, 2000).

In fungi, the MAPK cascade has been reported to be involved in regulating several essential developmental processes such as sporulation, mating, hyphal growth and pathogenicity (Dickman and Yarden, 1999; Xu, 2000). Genes involved in this cascade that have been characterised in fungi include *CHK1* (*Colletotrichum heterostrophus*) (Lev *et al.*, 1999), *CMK1* (*Colletotrichum lagenarium*) (Takano *et al.*, 2000), *PMK1* (*Magnaporthe grisea*) and *ubc3* (*Ustilago maydis*) (Mayorga and Gold, 1999). Alteration of these genes has been shown to affect pathogenicity, for instance mutants of *cmk1* were non-pathogenic and failed to form appressoria (Takano *et al.*, 2000); *chk1* mutants developed poor aerial hyphae, failed to produce conidia and had significantly reduced virulence (Lev *et al.*, 1999); *pmk1* were non-pathogenic, failed to cause blast lesions on compatible rice plants even when inoculated through wounds (Xu and Hamer, 1996); while those of *ubc3* were reduced in mating, formation of filamentous dikaryons, and virulence (Mayorga and Gold, 1999; Muller *et al.*, 1999).

A MAP kinase gene, *BMP1*, was recently identified in *B. cinerea* (Zheng *et al.*, 2000). This gene is 94% identical and 95% similar to *PMK1* of *M. grisea*. It also shows homology to the four other fungal MAP kinases listed above in addition to *FsMAPK* from *Nectria haematococca* (Zheng *et al.*, 2000). Deletion mutants of *bmp1* form typical greyish colonies and produce abundant quantities of normal macroconidia and microconidia. Macroconidia resuspended in glucose solution did not show any reduction or delay in conidial germination, however a reduction in vegetative growth was observed. Infection of tomato leaves, carnation and rose flowers with conidia from *bmp1* mutants did not lead to development of necrotic lesions even after prolonged incubation. In addition, the mutants were also less pathogenic on wounded carnation flowers than wild-type, an indication that *BMP1* is essential for appresorium formation and invasive growth. Scanning electron microscopy revealed that *bmp1* mutant conidia germinated and formed germ tubes but never penetrated which indicated that *BMP1* is not essential for fungal growth or conidiation but necessary for fungal pathogenesis (Zheng *et al.*, 2000).

Another MAP kinase gene, *Bcsak1*, that encodes a stress-activated protein kinase (SAPK) was recently cloned and characterised in *B. cinerea* (strain ATCC 58025) (Segmüller *et al.*, 2007). Stress-activated protein kinases are MAP kinases that specifically transmit environmental stress signals. Members of this subfamily have been identified in other organisms such as in mammals and yeasts. In these organisms, SAPKs are involved in resistance to hyperosmotic stress, heat shock, UV light irradiation and oxidative stress (Degols *et al.*, 1996; Kato *et al.*, 1996; Kyriakis and Avruch, 1996). In pathogenic fungi such as *Cryphonectria parasitica* and *Bipolaris oryzae*, SAPKs are involved in conidiation, virulence and resistance to osmotic, oxidative and UV stress. The SAPK identified in *B. cinerea* shows significant similarity to SAPKs from other filamentous fungi such as MapIII (90%) from *Blumeria graminis*, CpMK1 (89%) from *C. parasitica*, Osm1 (88%) from *M. grisea*, and Os-2 (88%) from *Neurospora crassa*. Deletion of this gene led to a complete lack of conidiation, increased production of microconidia and sclerotia, and increased resistance to the fungicide iprodione (dicarbomixide). The mutants were also sensitive to hyperosmotic stress caused by sodium chloride, oxidative stress caused by hydrogen peroxide (H_2O_2) and to the fungicides dicloran (aromatic hydrocarbon) and fludioxonil (phenylpyrrol). *BcSAK1* is also essential for pathogenicity as mutants could not infect intact plants or colonize wounded tissue. Another kinase, *Bos1*, shown to encode a class III histidine kinase was recently identified in *B. cinerea* and shown to mediate resistance to the fungicides iprodione, fludioxonil and quintozene (aromatic hydrocarbon). It was also shown to be necessary for for normal asexual development and virulence (Cui *et al.*, 2002; Viaud *et al.*, 2006).

1.7.2 cAMP signalling

The cAMP signalling cascade involves G protein-coupled receptors (GPCRs), heterotrimeric G protein subunits (Gα, Gβ and Gγ), adenylate cyclase and cAMP dependent protein kinase, (protein kinase A, PKA). Signal molecules targeted to the cell bind at the cell surface to receptors, the GPCRs, embedded in the plasma membrane triggering an allosteric change in these receptors. GPCRs are transmembrane with the N terminus external to the cell while the C terminus extends into the cytoplasm (Li *et al.*, 2007). They interact with the intracellular heterotrimeric

G proteins. Signals are passed on to the intracellular G-proteins triggering an allosteric change in Gα, the G protein subunit responsible for activation of cAMP formation. This causes the nucleotide binding site on Gα to become more accessible to the cytosol, where the concentration of guanosine 5'-triphosphate (GTP) is higher than that of guanosine 5'-diphosphate (GDP). Substitution of GTP for GDP causes another allosteric change in Gα dissociating it from the inhibitory $\beta\gamma$ subunit complex. The availability of Gα then stimulates adenylate cyclase which catalyzes the synthesis of cAMP from ATP. However, it has been shown recently that G$\beta\gamma$ can also stimulate adenylate cyclase. The best defined target of cAMP is PKA which mediates most if not all physiological effects of cAMP in eukaryotes including fungi. Cyclic AMP activates PKA by binding to its regulatory subunit which induces conformational changes that cause dissociation of the tetramer into dimeric regulatory subunits and active monomeric catalytic subunits. Liberated catalytic subunits catalyze the phosphorylation of serine or threonine residues on target proteins which may include metabolic enzymes and transcription factors (Daniel *et al.*, 1998; Rich and Karpen, 2002; Neves *et al.*, 2002; Janetopoulos *et al.*, 2001; Li *et al.*, 2007).

Components of the cAMP cascade have been identified in various pathogenic fungi and shown to have a role in pathogenicity. For instance, one of the two genes encoding G protein α subunits, *CPG1* in *C. parasitica* is required for both virulence and female fertility (Gao and Nuss, 1996). Of the three Gα encoding genes in *M. grisea*, *MAGB* is required for appresorium formation and perithecium production (Liu and Dean, 1997). In *C. heterostrophus*, a Gα encoding gene, *CGA1*, is required for appresorium formation and mating (Horwitz *et al.*, 1999) while in *U. maydis*, a mutation in one of the four Gα encoding genes *GPA3* causes mating deficiency and an inability to infect the host plant (Regenfelder *et al.*, 1997). In *Colletotrichum trifolii*, a Gα subunit encoding gene *CTG1* is involved in conidial germination and appresorium formation (Truesdell *et al.*, 2000).

Recently a GPCR encoding gene *BTP1* was identified in *B. cinerea* (Schulze Gronover *et al.*, 2005). Targeted gene replacement did not result in reduced pathogenicity or sensitivity to chemical stress however, it affected the expression of genes encoding glutathione S-transferases (GSTs). Genes encoding Gα subunits (*BCG1*, *BCG2* and *BCG3*) were also recently characterised (Schulze Gronover

et al., 2001; Doehlemann *et al.*, 2006). *BCG1*, *BCG2* and *BCG3* belong to subgroup I, II and III of fungal Gα proteins respectively. *BCG1* is 48, 95 and 96% identical with *BCG2*, *MAGB* from *M. grisea* and *CPG1* from *C. parasitica* respectively (Schulze Gronover *et al.*, 2001). *BCG3* is less similar to *BCG1* (49%) and *BCG2* (42%) but more similar to *MAGA* from *M. grisea* (78%), *GANB* from *Aspergillus nidulans* (80%) and *GASC* from *Penicillium marneffei* (79%) (Doehlemann *et al.*, 2006). *BCG1* mutants could sporulate, germinate and were able to penetrate intact bean and tomato leaves, however invasion of plant tissue stopped at primary lesions and the pathogen could not secrete proteases (Schulze Gronover *et al.*, 2001). Alteration of *BCG2* had no obvious effect on growth, conidiation, or protease secretion, however its loss led to significant retardation of fungal development (Schulze Gronover *et al.*, 2001).

Mutants of *BCG3* produced less conidia and large amounts of aerial mycelium. Conidia of these mutants showed normal germination on leaf surfaces but penetrated less efficiently. They also produced a large number of sclerotia under conditions which do not normally lead to sclerotium formation. Exogenous application of cAMP completely restored wild-type sporulation and suppressed sclerotium formation (Doehlemann *et al.*, 2006). These data indicated that the Gα subunits have a number of functions with *BCG1* regulating growth rate and colony morphology, colonization of host tissue, expression and secretion of hydrolytic enzymes that are involved in plant penetration (Schulze Gronover *et al.*, 2001). *BCG2* on the other hand is required for normal colonization (Schulze Gronover *et al.*, 2001) while *BCG3* is required for carbon source-induced germination together with adenylate cyclase/cAMP and *BMP1* (Doehlemann *et al.*, 2006). Its is also necessary for efficient primary lesion formation by germinated conidia but not for pathogenic growth in expanding lesions.

Genes encoding another component of the cAMP cascade, adenylate cyclase, have also been characterised in various fungi such as *U. maydis* (*UAC1*), and *M. grisea* (*MAC1*). *UAC1* mutants show constitutive filamentous growth while those of *MAC1* have reduced vegetative growth and conidiation, however both mutants are non-pathogenic. Defects in both mutants can be restored by exogenous application of cAMP or suppressor mutations in the regulatory subunit of PKA (Gold *et al.*, 1997;

Adachi and Hamer, 1998). An adenylate cyclase gene *BAC1* has been identified in *B. cinerea* (Klimpel *et al.*, 2002). The sequence of *BAC1* has similar characteristic domains as those described for other fungal adenylate cyclases including a putative Ras-binding motif (RA), leucine rich repeat (LRR) motifs, a region with sequence homology to Mn^{2+} and Mg^{2+} dependent protein serine/threonine phosphatases of the PP2C phosphatase family, the highly conserved catalytic domain, and a putative CAP-binding domain. Mutants created by altering this gene could penetrate host tissue with the same efficiency but at a much slower rate than the wild-type. Resulting secondary lesions were much smaller in diameter and no conidia were produced from infected leaves. Doehlemann *et al.* (2006) also reported that *bac* mutants were defective in carbon source-induced germination on glass but germinated normally on hydrophobic surfaces.

1.7.3 Ca^{2+}-calmodulin signalling

The involvement of the Ca^{2+}-calmodulin-dependent signalling pathway in *B. cinerea* pathogenesis has also been investigated. This pathway is initiated by a rise in the concentration of intracellular Ca^{2+} which may occur as a response to different signals. This is followed by binding of Ca^{2+} to calmodulin which subsequently leads to its activation. Calmodulin then activates a number of target enzymes such as calmodulin-dependent protein kinases and phosphatases such as calcineurin (CaN). CaN acts as an effector of Ca^{2+} signalling by regulating the phosphorylation state of proteins. Calcineurin is a heterodimer composed of a catalytic A subunit (CaNA) and a calcium-binding regulatory B subunit (CaNB) and belongs to a superfamily of protein serine/threonine phosphatases, and is regulated by Ca^{2+}. It is the only protein phosphatase dependent on Ca^{2+} and calmodulin for its activity thereby making it one of the most common intracellular transducers of Ca^{2+} signalling pathways (Olson and Williams, 2000; Crabtree, 2001).

The role of CaN has been elucidated in most fungi; in *A. nidulans* (Rasmussen *et al.*, 1994) and *N. crassa* (Kothe and Free, 1998), it was shown to be involved in hyphal branching, changes of hyphal morphology and concomitant loss of the distinctive tip-high calcium gradient, while in *M. grisea* it was shown to be involved in appresorial infection structure formation (Lee and Lee, 1998; Viaud

et al., 2002). Inhibitors of CaN have been suggested to have strong antifungal activity (Lengeler *et al.*, 2000). The immunosuppressive drug cyclosporin A (CsA) is toxic to many pathogenic fungi (Tropschug *et al.*, 1989). CsA acts by binding to cyclophilin A (CyPA) forming a complex that targets and inhibits CaN (Liu *et al.*, 1991). CyPA is an example of a conserved family of proteins, the cyclophilins, present in many organisms and which have been shown to mediate a number of cellular processes (Marks, 1996). Another immunosuppressant with toxic effects to fungi is FK506 which binds to the immunophilin FKBP12 (Liu *et al.*, 1991).

The involvement of cyclophilins in pathogenicity has been elucidated in a number of pathogens. For example, cyclophilin proteins CPA1 and CPA2 in *C. neoformans* play a vital role in cell growth and virulence (Wang *et al.*, 2001) while in *M. grisea* the cyclophilin CYP1 regulates virulence related functions including appresorium turgor generation and lipid biosynthesis (Viaud *et al.*, 2002). A cyclophilin encoding gene *BCP1* was recently identified in *B. cinerea* (Viaud *et al.*, 2003). Null mutants of this gene were not altered in vegetative growth, conidial germination or conidiogenesis. The mutant expressed reduced pathogenicity on bean and tomato leaves and complementation restored full pathogenicity in the mutants indicating that BCP1 is a virulence determinant. The fact that the mutants were not altered in the infection structure development suggested that this gene is involved in latter stages of plant infection.

Addition of CsA to medium led to inhibition of vegetative growth and development of infection structures in the wild-type strain but not in the mutants, an indication that CsA activity requires the BCP1 cyclophilin and CaN seems to be involved in *B. cinerea* hyphal morphology and growth. A macroarray involving 2,839 *B. cinerea* genes and comparing CsA-treated and untreated mycelia led to identification of 18 calcineurin-dependent (*CND*) genes. Three of the co-regulated *CND* genes were organized as a physical cluster that could be involved in secondary metabolism. The signature of BCP1 inactivation was also studied by comparing gene expression in the wild-type and the *bcp1* mutant. This resulted in the identification of three *BCP1* cyclophilin-dependent genes that were different from the *CND* genes.

1.7.4 Ras and Rho GTPases signalling

The role of Ras and Rho GTPases has been investigated in a number of pathogens. For example, in *A. nidulans*, RasA GTPase in conjunction with the cAMP and PKA have been shown to control the germination process (Fillinger *et al.*, 2002); in *Saccharomyces cerevisiae*, Ras1 and Ras2 senses changes in the extracellular environment and regulate cAMP synthesis and cell cycle progression. Ras2 also regulates filamentous and invasive growth via both the MAP kinase and cAMP pathways (Lengeler *et al.*, 2000) while Ras and Rho GTPases are coordinately involved in the regulation of cell polarity and germination in *Penicillium marneffei* (Boyce *et al.*, 2005). The involvement of Ras and Rho GTPases in pathogenicity of *B. cinerea* is also currently under investigation (Williamson *et al.*, 2007).

1.7.5 Penetration

Upon conidial germination, penetration of the intact host surface is in most cases achieved directly, although penetration through wounds may be opportunistically employed by the fungus. Direct penetration presents the fungus with two challenges in form of the plant surface as well as the cell wall. Besides protecting tissues against a relatively dry atmosphere by minimizing non-stomatal water loss, plant surfaces also provide the first line of defence against pathogenic organisms. The primary structure of the plant surface is the cuticle, which is composed of two separate layers, an inner portion characterised by intracuticular wax associated with a polyester matrix of cutin, and a continuous surface layer of epicuticular wax without cutin (Jeffree, 2006). Cutin is a polymer composed of mainly C16 and C18 hydroxy fatty acids which are predominantly linked by ester bonds. Wax is a complex mixture of very-long-chain fatty acids, hydrocarbons, alcohols, aldehydes, ketones, esters, triterpenes, sterols, and flavonoids (Post-Beittenmiller, 1996).

The second challenge is the cell wall; it is composed of cellulose, hemicellulose, pectin, lignin and proteins (Vorwerk *et al.*, 2004). Cellulose is composed of chains of β-1,4-linked glucose that are hydrogen bonded to form an insoluble and inelastic crystalline material. The cellulose fibres are interconnected with hemicellulose molecules (e.g. arabinoxylan or xyloglucan) that are hydrogen bonded to the

surface of the cellulose microfibrils and embedded in a matrix of pectin. Arabinoxylan is present in cell walls of cereals and hard woods while xyloglucan is present in those of dicotyledonous and some monocotyledonous plants (e.g. onion).

The main component of pectin is rhamnogalacturonan I (RGI) which consists of a backbone of alternating rhamnose and galacturonic acid with various side groups such as galactans and arabinans while the other components are homogalacturonan (HG), xylogalacturonan (XGA) and rhamnogalacturonan II (RGII) whose main backbone is 1,4-linked galacturonic acid. Plant cell wall proteins are typically glycoproteins which are rich in the amino acids hydroxyproline, proline, and glycine (Elad, 1997). Therefore *B. cinerea* produces an array of hydrolytic enzymes aimed at attacking all of these plant preformed structural defences. Because the cuticle forms the first barrier that fungal pathogens must breach, the enzyme cutinase has always been thought to play a pathogenic role most likely that of aiding direct penetration of host cuticle.

Cutinase acts by hydrolyzing ester bonds which degrades the cuticle into component fatty acids. *In vitro* production of this enzyme during growth on cutin has been reported in a number of plant pathogenic fungal species (Ettinger *et al.*, 1987; Trail and Köller, 1990). Evidence for its role in enzyme-mediated penetration has come from a number of studies. For example, treating gerbera flowers with antibodies raised against cutinase, Salinas *et al.* (1986) were able to observe an 80% reduction in lesion formation after infection with *B. cinerea*. Production of cutinase is induced by cutin, cutin hydroxylase and cutin monomers such as 16-hydroxyhexadecanoic acid (van der Vlugt-Bergmans *et al.*, 1997). A *cutinase A* (*cutA*) gene from *B. cinerea* has been isolated and cloned (van der Vlugt-Bergmans *et al.*, 1997).

In vitro expression of this gene is induced by 16-hydroxyhexadecanoic acid (van Kan *et al.*, 1997; van der Vlugt-Bergmans *et al.*, 1997) and *in planta* on tomato leaves (van Kan *et al.*, 1997; van der Vlugt-Bergmans *et al.*, 1997) and gerbera flowers (van Kan *et al.*, 1997). *CutA*-deficient mutants did not show any differences in lesion development or morphology of fungal penetration structures compared to the wild-type strain. This indicated that cutA does not play any role and is therefore not required during penetration of gerbera flowers and tomato leaves. In the

study by van der Vlugt-Bergmans *et al.* (1997), *cutA* transcript was expressed in *B. cinerea* infected leaves until the stage of sporulation indicating that this gene is not only produced during the early stages of the infection process. The implication of this was that this gene might play a role in the degradation of cutin during the saprophytic growth phase of the fungus which occurs in the final stages of plant colonization (van der Vlugt-Bergmans *et al.*, 1997). The lack of a role in penetration by cutA suggests that *B. cinerea* employs other hydrolytic enzymes to attack the host epidermis.

The other enzymes that may be employed by *B. cinerea* during penetration are lipases and phospholipases (Elad and Evensen, 1995). The involvement of a lipase (EC 3.1.1.3) in the penetration process was demonstrated by Commenil *et al.* (1999) who showed that this enzyme was produced in the early stages of infection of grapevine leaves. In an earlier experiment, they also demonstrated that inhibition of enzyme activity by antilipase antibodies prevented infection of the tomato leaf tissues by *B. cinerea* (Commenil *et al.*, 1998). Expression of this enzyme was also induced by wax esters and free fatty acids but not oleanolic acid which supports the hypothesis that some wax components may act as signals to the fungus to induce lipase production and excretion. In addition to penetration of the host cuticle, this lipase may possibly be involved in surface recognition or adhesion. The amino acid composition of this enzyme is enriched in hydrophobic amino acids such as alanine, glycine, and leucine which supports the idea that it may play a role in the immediate adhesion of *B. cinerea* on host hydrophobic surfaces.

To clarify its role in pathogenicity, Reis *et al.* (2005) re-isolated the gene using primers based on the partial amino acid sequence obtained from the purified lipase (Commenil *et al.*, 1999). The corresponding gene (*lip1*) was cloned. Its sequence was 50-60% and 35-45% identical to putative lipases from filamentous fungi and yeasts (*Candida rugosa* and *Geotrichum candidum*). The extent of lesion formation by knockout mutants was the same as for the wild-type strain, an indication that *lip1* is not required for host penetration. Because disruption mutants of *lip1* and *cutA* (van Kan *et al.*, 1997) were not affected in their pathogenicity, a double mutant *lip1cutA* was constructed. Esterase activity of the double mutant was tested and found to be present but at very low levels. Lesion formation induced by double

mutants was not significantly different from that induced by the wild-type strain on tomato and bean leaves as well as carnation and gerbera flowers.

Because of the presence of esterase activity in the double mutants, the authors postulated that the lack of phenotype could be due to the presence of other cutinolytic enzymes that are secreted by *B. cinerea* germlings. Ungerminated conidia were observed to have esterase activity which was of cell wall origin. The enzyme was not encoded for by *lip1* or *cutA* because it was fully present in the knockout mutants. This enzyme activity decreased during germination, an indication that no newly synthesized esterase was present in germ tube walls. It was also unlikely that an immobilized enzyme activity restricted to the conidial wall could contribute to the penetration process. It has therefore been suggested that *B. cinerea* breaches the host cuticle mainly by physical forces generated by the turgor of the penetrating hyphal tip rather than by enzyme dissolution. This suggestion is supported by recent studies carried out on the *B. cinerea BMPI* gene and the tetraspanin encoding gene *BcPLS1* (Gourgues *et al.*, 2004).

Tetraspanins are animal proteins involved in membrane complexes that control cell adhesion, differentiation, and motility (Hemler, 2003). They were first identified in mammals but have now been identified in many other organisms including fishes, worms, insects, fungi and even protozoans (Veneault-Fourrey *et al.*, 2006). In fungi studied so far, they have been shown to play a significant role in penetration of host tissue. The tetraspanin gene *BcPLS1* identified in *B. cinerea* is orthologous to *MgPLS1* of *M. grisea* (Clergeot *et al.*, 2001) and *ClPLS1* of *Colletotrichum lindemuthianum* (Veneault-Fourrey *et al.*, 2005). Mutants of *BcPLS1* created through targeted gene replacement were unable to infect tomato and bean leaves and gherkin cotyledons even after 4 days. Introduction of the wild-type *BcPLS1* restored pathogenicity of the mutants.

Inoculation of wounded tomato and bean leaves as well as rose flowers with a conidial suspension of *Bcpls1* mutants resulted in spreading lesions similar to those of the wild-type. Cytological analysis of the penetration process showed that *Bcpls1* mutant conidia germinated and differentiated appresorium with the same kinetics as the wild-type but these appresorium were unable to direct successful penetration events. All these studies were backed up with expression studies showing that

BcPLS1 expression was restricted to the penetration process, starting during spore germination and decreasing after completion of penetration. Taken together these results indicated that *BcPLS1* is essential for pathogenicity on intact tissue irrespective of the host but is not required for pathogenicity on wounded plants (Gourgues *et al.*, 2004). Disruption of both genes in *M. grisea* and *C. lindemuthianum* resulted in similar observations.

1.7.6 Cell wall degrading enzymes

Following penetration, fungi produce a battery of cell wall degrading enzymes (CWDEs) which are aimed at degrading components of the cell wall (Elad, 1997). These enzymes are produced shortly after germination and include pectin methylesterases, polygalacturonases and pectate and pectin lyases (Elad, 1997: Reignault *et al.*, 2008). Pectin methylesterase (EC 1.1.11) de-esterifys pectin into methanol and polygalacturonic acid (PGA). De-esterification by pectin methylesterase allows subsequent action of depolymerizing enzymes such as polygalacturonases (endo (EC 3.2.1.15) and exo (EC 4.2.1.67 and EC 4.2.1.82)) which cleave glycosidic bonds by hydrolysis and pectate lyases (endo (EC 4.2.2.2) and exo (EC 4.2.2.9)) which break PGA into oligogalacturonides (OGAs) by β-elimination. In the alternative pectin degradation pathway, pectin lyase (EC 4.2.2.10) is able to depolymerize native pectin via β-elimination into methylated galacturonide residues. Some of these enzymes have been identified in *B. cinerea* infected tissues from many plants.

Recently a gene encoding a pectin methylesterase (*Bcpme1*) was identified in *B. cinerea* and cloned (Valette-Collet *et al.*, 2003). A mutant created by disruption of this gene had reduced ability to grow on pectin-containing medium *in vitro* and *in planta* when compared to the wild-type. Complementation of the *Bcpme1* mutation restored the wild-type phenotype. Pathogenicity tests showed reduced rotting activity of the mutant on *A. thaliana*, grapevine (*Vitis vinifera*) leaves and apple fruit (*Malus domestica*). In addition, PME activity was 75% lower in the mutant than in the wild-type. Therefore pectin methylesterase activity encoded by *Bcpme1* is a key virulence factor in *B. cinerea* pathogenicity. Two other mutants, one of which lacked the *Bcpme1* gene already investigated by Valette-Collet *et al.*

(2003) while the other lacked the *Bcpme2* gene, were recently obtained by Kars *et al.* (2005b) by gene disruption through PCR-based targeted mutagenesis but from a distinct wild-type strain. These mutants were not affected in their *in vitro* growth on a pectin-rich medium or their pathogenicity on tomato and grapevine leaves. Kars *et al.* (2005b) attributed this to strain-specific importance of pectin methylesterase, an observation that had been made by Siewers *et al.* (2005) on the botrydial toxin.

B. cinerea also possesses a large family of endopolygalacturonase encoding genes. Genes isolated and cloned by Wubben *et al.* (1999) included *Bcpg1*, *Bcpg2*, *Bcpg3*, *Bcpg4*, *Bcpg5*, and *Bcpg6*. These genes were shown to be differentially expressed when the fungus was grown in liquid culture on different carbon sources (Wubben *et al.*, 2000). A basic constitutive expression was observed for two genes *Bcpg1* and *Bcpg2*. The genes *Bcpg4* and *Bcpg6* were induced by galacturonic acid while low pH of the medium resulted in induction of *Bcpg3*. Although *Bcpg5* was inducible, the factors responsible for its induction could not be identified. Galacturonic acid-induced expression of *Bcpg4* gene was repressed by the presence of a more favourable carbon source such as glucose.

The expression of this set of genes was also investigated by ten Have *et al.* (2001) on four different host plants; tomato, broad bean, apple and zucchini. All six genes were still shown to be differentially expressed depending on the infected host. The gene *Bcpg1* was expressed early in all the tested tissues. Like *Bcpg1*, *Bcpg2* was also detected early in all plants but not apple fruit. *Bcpg3* and *Bcpg5* were expressed in apple tissue only. Because *Bcpg1* and *Bcpg2* are among the earliest expressed genes during infection, it is possible that their gene products have a significant role in the early stages of the infection process. This supposition is supported by the fact that disruption mutants of these two genes *Bcpg1* (ten Have *et al.*, 1998) and *Bcpg2* (Kars *et al.*, 2005a) show a severe reduction in virulence on tomato leaves for *Bcpg1* and tomato and broad bean leaves for *Bcpg2*.

Polygalacturonases, pectin lyases, and pectate lyases fragment the homogalacturonan regions of pectin but can not degrade rhamnogalacturonan (De Vries and Visser, 2001). Rhamnogalacturonan is acted on by rhamnogalacturonan-degrading enzymes (RGDEs) such as rhamnogalacturonan acetyl esterase which removes acetyl residues followed by rhamnogalacturonan hydrolase which completely

hydrolyzes the rhamnogalacturonan backbone (De Vries and Visser, 2001). RGDEs have been reported in pathogenic fungi such as *Aspergillus aculeatus* (Suykerbuyk *et al.*, 1996) and *Aspergillus niger* (Suykerbuyk *et al.*, 1997). In *A. aculeatus*, expression of a rhamnogalacturonan hydrolase was induced by pectin or a combination of two constituent monosaccharides of pectin, rhamnose and galacturonic acid. A rhamnogalacturonan hydrolase (*RGase A*) encoding gene was cloned in *B. cinerea* (Chen *et al.*, 1997). Expression of this gene was induced by apple pectin, RGI and glucose and was not affected by catabolite repression. The role of this enzyme in pathogenicity was not determined.

Cellulases are also known to play a role in cell wall degradation. They catalyze the degradation of β-1,4 glycosidic bonds in the cellulose polymer (Lynd *et al.*, 2002). The complete degradation of cellulose is carried out by four different types of this enzyme; endo-β-1,4-glucanases (EC 3.2.1.4) cleave internal glycosidic bonds leaving shorter polysaccharide chains, cellobiohydrolases (EC 3.2.1.91) release cellobiose from non-reducing ends of cellulose, exo-β-1,4-glucanases (EC 3.2.1.74), liberate successive glucose units from the polymer ends and finally β-glucosidases (EC 3.2.1.12) which hydrolyze cellobiose to glucose. A gene *cel5A* encoding a endo-β-1,4-glucanase was recently isolated and cloned in *B. cinerea* (Espino *et al.*, 2005). Lesion sizes observed for *cel5A* disruption mutants were not different from those of the wild-type. Although no significant role in pathogenicity can be attached to *cel5A*, it was expressed during infection of tomato leaves by *B. cinerea*. Expression of the transcript was observed to increase continuously for the first two days. The authors postulated that *cel5A* might be more involved in producing nutrients by macerating tissue than facilitating the infection process through softening plant tissues.

Aspartic proteinases are other enzymes that are involved in degradation of plant cell walls targeting cell wall proteins. These enzymes have been reported in a number of pathogenic fungi such as *Colletotrichum gloeosporioides* (Clark *et al.*, 1997), *Rhizopus oryzae* (Farley and Sullivan, 1998), *Cochliobolus carbonum* (Murphy and Walton, 1996), *Phytophthora infestans* (Paris and Lamattina, 1999), *Sclerotinia sclerotiorum* (Poussereau *et al.*, 2001) as well as *B. cinerea* (Elad and Evensen, 1995). Movahedi and Heale (1990) demonstrated that treating carrot cell wall preparations with an aspartic proteinase isolated from *B. cinerea* resulted in reduced protein

content which subsequently facilitated release of galacturonic acid by pectin lyase. In addition, infection of several fruits and vegetables by *B. cinerea* was reduced by adding pepstatin, an aspartic proteinase inhibitor. Both these scenarios show that aspartic proteinases play a significant role in pathogenesis of *B. cinerea*. *B. cinerea* genes encoding these enzymes (*Bcap1*, *Bcap2*, *Bcap3*, *Bcap4*, *Bcap5*) were recently cloned and characterised, however their precise role in the pathogenesis has not yet been determined (ten Have *et al.*, 2004).

1.7.7 Active oxygen species

B. cinerea produces toxic levels of active oxygen species (AOS) as pathogenicity factors to effectively colonize host tissue. When plants are attacked by avirulent pathogens, one of the earliest responses following pathogen recognition is rapid tissue necrosis at the site of infection, a phenomenon referred to as the hypersensitive response (HR) (Mehdy, 1994). The HR is a type of programmed cell death but differs from cell death by necrosis because it requires active plant metabolism and depends on activity of the host transcriptional machinery (He *et al.*, 1994; Dangl *et al.*, 1996). At the site of, and in cells surrounding, the HR, one of the earliest observed events is an oxidative burst (Doke, 1985; Dixon *et al.*, 1994; Mehdy, 1994; Baker and Orlandi, 1995; Grant and Loake, 2000).

The oxidative burst is characterised by the generation of AOS, such as the hydroxyl radical ($OH^{\cdot}$), superoxide ion (O_2^-) and its dismutation product, H_2O_2 (Levine *et al.*, 1994; Lamb and Dixon, 1997; Tiedemann, 1997; Grant and Loake, 2000). The superoxide ion and hydroxyl radical are very unstable, however, the relatively stable H_2O_2 can easily cross membranes and be converted into the highly reactive and thus very unstable hydroxyl radical by a fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^{\cdot}$) (Deighton *et al.*, 1999). In addition to reacting with carbohydrates, proteins or DNA, hydroxyl ions can also destroy cell integrity by initiating a chain reaction of lipid peroxidation. AOS are also referred to as reactive oxygen species (ROS) (Pitzschke *et al.*, 2006) or reactive oxygen intermediates (ROI) (Liszkay *et al.*, 2004).

Most of the AOS generated during the HR are produced by NADPH oxidase also referred to as respiratory burst oxidase, (RBO) (Torres *et al.*, 2005). This enzyme was first described in mammalian neutrophils as a multicomponent complex mediating microbial killing (Lambeth, 2004). The enzymatic subunit of NADPH oxidase $gp91^{phox}$ is concerned with the transfer of electrons to molecular oxygen to generate superoxide. Genes homologous to the *respiratory burst oxidase* (*rboh*) have been identified in several plant species including *Arabidopsis*, potato, rice and tomato (Groom *et al.*, 1996; Torres *et al.*, 1998; Keller *et al.*, 1998; Amicucci *et al.*, 1999; Simon-Plas *et al.*, 2002; Yoshioka *et al.*, 2001, 2003). In *Arabidopsis*, ten *rboh* genes were identified (*AtrbohA-AtrbohJ*) (Torres *et al.*, 2005). Members of this family have been shown to mediate production of apoplastic AOS in response to attack by pathogens and also during abiotic, environmental and developmental cues (Torres and Dangl, 2005). Other enzymes that have been implicated in the production of AOS include glucose oxidase, lipoxygenase, xanthine oxidase and cell wall peroxidases (Lamb and Dixon, 1997; Grant and Loake, 2000; Bolwell *et al.*, 1998; Mayer *et al.*, 2001; Torres *et al.*, 2002b).

The AOS generated during the oxidative burst are thought to enhance disease resistance against biotrophic pathogens in a number of ways. First, they may result in cross-linking of cell wall proteins, rendering the cell wall more resistant to attack by fungal enzymes. Second, they may act as second messengers in the activation of the HR in the plant and or the induction of defence genes. Third, elevated levels of AOS might be actively involved in killing pathogen and or host cells during the HR (Lamb and Dixon, 1997). However, (Torres *et al.*, 2002a) have demonstrated that AOS can alternatively function as negative regulators of HR during plant defence. The *Arabidopsis atrbohf* mutant and *atrbohd atrbohf* double mutant show enhanced HR after infection with *Hyaloperonospora parasitica* Emco5.

The HR has been shown to significantly reduce continued colonization by biotrophic pathogens however, it does not deter continued colonization by necrotrophic pathogens such as *B. cinerea*, in fact they opportunistically exploit it to promote host cell death (Govrin and Levine, 2000). The role of AOS in *B. cinerea* colonization of plant tissue is supported by a number of studies. While working on *Arabidopsis*, Govrin and Levine (2000) found that elevation of H_2O_2 lev-

els or other AOS *in planta* led to increased colonization of host tissue by *B. cinerea*. Infection of bean and tomato leaves by *B. cinerea* resulted in massive accumulation of H_2O_2, both in the plasma membrane and extracellular sheath covering the surface of the fungal hyphae (Tenberge *et al.*, 2002; Schouten *et al.*, 2002). Studies using electron paramagnetic resonance spectroscopy have also shown that infection with *B. cinerea* leads to accumulation of free radicals in and around the spreading lesion (Muckenschnabel *et al.*, 2001, 2003). This not only results in lipid peroxidation (Deighton *et al.*, 1999; Muckenschnabel *et al.*, 2001, 2002) but also the the depletion of antioxidants (Muckenschnabel *et al.*, 2002). Because of the deleterious effects of AOS, necrotrophs like *B. cinerea* protect themselves from oxidative damage with aid of a glucan sheath present on the surface of mycelium which is important for host-derived AOS (Gil-ad *et al.*, 2001). They also employ an antioxidant system composed of an array of AOS scavenging enzymes (Gil-ad *et al.*, 2000; Mayer *et al.*, 2001). Such enzymes include guaiacol peroxidase, glucose oxidase, ascorbic peroxidase, glutathione peroxidase, superoxide dismutase (SOD), laccase and catalase (Gil-ad *et al.*, 2000; Mayer *et al.*, 2001). Gil-ad *et al.* (2000) demonstrated that the level of AOS scavenging enzymes is determined by the conditions under which the fungus grows, an indication that *B. cinerea* is well equipped to suppress oxidative stress that may arise during host colonization.

Two catalase genes *Bccat1* (intracellular) (van der Vlugt-Bergmans *et al.*, 1997) and *Bccat2* (extracellular) (Schouten *et al.*, 2002) have been isolated and cloned from *B. cinerea*. Catalases mediate the enzymatic breakdown of H_2O_2 by converting it to molecular oxygen and water. Both of these genes were induced on addition of exogenous H_2O_2, however only *Bccat2* was observed to be expressed *in planta*. In the study by Schouten and associates, disruption mutants of *Bccat2* were created and found to be more sensitive to H_2O_2 than the wild-type strain. However, after prolonged incubation, they were able to grow on plates containing higher H_2O_2 concentrations. The increased sensitivity of *Bccat2* disruption mutants to H_2O_2 compared to wild-type illustrates that the *BCCAT2* protein provides a protective role to the fungus in infected plant tissue. Because *Bccat2* disruption mutants were able to cope with oxidative stress under high H_2O_2 concentrations after prolonged incubation, it is likely other stress-responsive genes may be employed by *B. cinerea*. In this study, the transcript of *Bccat1* was not detected indicating

that the intracellular catalase does not play a role in the detoxification of externally added H_2O_2 or compensation in the *Bccat2* deficient mutants.

Significantly higher transcript levels of *Bcgst1* were detected in the *Bccat2* deficient mutants as compared to the wild-type strain. This *B. cinerea* gene, *Bcgst1*, encodes a GST (Prins *et al.*, 2000a). Prins *et al.* (2000a) showed that the level of *Bcgst1* transcript increases in the presence of exogenous H_2O_2 as well as other stresses like high salt and cycloheximide. It is also expressed *in planta* at a level which is more or less proportional to fungal biomass during infection. Mutants created by disruption of the *Bcgst1* gene were not found to have reduced virulence compared to controls which indicates that this gene is not involved in the infection process on tomato leaves. Two *B. cinerea* genes, one encoding a Cu-Zn SOD (*Bcsod1*) and the other encoding a putative glucose oxidase (*Bcgod1*), have been cloned and characterised (Rolke *et al.*, 2004). The enzyme SOD catalyzes the dismutation of the superoxide ion to H_2O_2 and water. Deletion mutants of *bcgod1* displayed normal virulence on bean leaves, however *bcsod1* mutants showed a significantly retarded development of the lesion an indication that the Cu-Zn SOD activity is an important virulence factor in this interaction.

1.7.8 Detoxifying enzymes

B. cinerea produces enzymes aimed at detoxifying chemical defences within the plant that have antifungal activity. These chemical defences may be pre-existing (e.g. phytoanticipins) or induced in response to pathogen attack (e.g. phytoalexins) (Osbourn, 1996). Phytoanticipins exist in healthy plants in their biologically active forms and examples include the cyanogenic glycosides and glucosinolates (Morrissey and Osbourn, 1999). Phytoalexins on the other hand are synthesized from remote precursors in response to pathogen attack, probably as a result of *de novo* synthesis of enzymes (Morrissey and Osbourn, 1999). One of the enzymes produced by *B. cinerea* and known to detoxify these compounds especially phytoalexins, is laccase. It is an enzyme that catalyzes the oxidation of phenolic compounds together with the reduction of molecular oxygen into water (Messerschmidt and Huber, 1990). This enzyme was implicated in the detoxification of the grapevine phytoalexin resveratrol (Pezet and Hoang-Van, 1991; Adrian *et al.*, 1998; Breuil *et al.*, 1999).

Resveratrol is important in host resistance to this pathogen because its accumulation in some plants such as grapevine and peanut (*Arachis hypogaea*) has been shown to correlate with increased resistance (Sbaghi *et al.*, 1995). Recently, two laccase genes, *Bclcc1* and *Bclcc2*, were isolated and characterised in *B. cinerea* (Schouten *et al.*, 2002). Unlike *Bclcc1*, *Bclcc2* was induced by tannic acid and resveratrol, an indication that it plays a more active role in the oxidation of both these compounds than *Bclcc1*. The role of the two genes in the oxidation of tannin and resveratrol was further confirmed in mutants that were deficient in the two genes. While all *Bclcc1* mutants remained like the wild-type strain capable of converting tannic acid and resveratrol, *Bclcc2* mutants lost the capability to convert the two compounds. The presence of other laccase genes in *B. cinerea*, like *Bclcc1*, is not enough to functionally complement the absence of *Bclcc2*.

Studies by Schoonbeek *et al.* (2001) also revealed that *B. cinerea* can employ an ATP binding cassette protein to detoxify resveratrol. The *B. cinerea* gene (*BcatrB*) encoding this protein was cloned and characterised. Its transcription was shown to be induced by resveratrol and another phytoalexin pisatin from peas. It is also induced in response to the phenylpyrrole fungicide fenpiclonil. Replacement mutants of this gene showed increased sensitivity to resveratrol compared to the wild-type strain, however no difference in sensitivity to pisatin was observed. These mutants also exhibited a small but significant increase in sensitivity to the fungicide fenpiclonil (phenylpyrrole) but no increase in sensitivity to cycloheximide (antibiotic) and the fungicides, vinclozolin (dicarboximide) and imazalil (azole). These results indicated that this ABC transporter is employed by *B. cinerea* to protect it against resveratrol in addition to the fungicide fenpiclonil. *B. cinerea* has also been shown to have the ability to detoxify phytoantipicins like the tomato steroidal glycoalkaloid saponin α-tomatine (Quidde *et al.*, 1998). *B. cinerea* achieves this by deglycosylating α-tomatine to β1-tomatine. A strain M3, which lacks this activity was non-pathogenic on tomato.

1.7.9 Toxins

B. cinerea produces a number of secondary metabolites that have phytotoxic activity and most of these have been identified in culture filtrates (Collado *et al.*, 2000). One of the phytotoxic metabolites that has been shown to be important in *B. cinerea* pathogenesis is botrydial. Studies by Deighton *et al.* (2001) demonstrated that this metabolite is produced in plant tissue infected with *B. cinerea*. Colmenares *et al.* (2002) showed that botrydial affects different crops and genotypes, an indication that it may act as a nonhost specific toxin. Recently, the pathway involved in the biosynthesis of botrydial was elucidated and the gene *Bcbot1* characterised. This gene was shown to be highly expressed in botrydial producing culture and in all wild-type strains, however, no expression was observed in *bcg1* mutants indicating that it is under control of *BCG1* (Schulze Gronover *et al.*, 2001). Knockout mutants of *Bcbot1* differed in virulence depending on the recipient strain. Virulence of a mutant that was less pathogenic on bean and tomato leaves was restored after complementation with *Bcbot1* indicating that the absence of *Bcbot1* was responsible for the mutant phenotype.

Oxalic acid is another non-host specific toxin used by necrotrophic pathogens to facilitate host cell death. One necrotroph known to produce this toxin in large amounts causing wilting in many infected plants is *S. sclerotiorum* (Hegedus and Rimmer, 2005). Guimaraes and Stotz (2004) demonstrated that oxalic acid promotes wilting through interfering with stomatal closure. This is achieved in two ways; oxalic acid stimulates the accumulation of potassium and starch hydrolysis in the guard cells and also disrupts the abscisic acid-dependent stomatal closure process. Oxalic acid may also play a role in the degradation of plant cell walls during pathogenesis by necrotrophs. It has been postulated that oxalic acid lowers the pH within the environment of the middle lamella and also sequesters calcium ions that are bound to pectins (Dutton and Evans, 1996; Hegedus and Rimmer, 2005). The low pH environment not only creates optimum conditions for fungal CWDEs but may also disrupt the molecular interaction between pathogen polygalacturonases and plant polygalacturonase-inhibiting proteins (PGIPs) allowing these enzymes to avoid inactivation (Cotton *et al.*, 2003; Favaron *et al.*, 2004). *S. sclerotiorum* mutants unable to secrete oxalic acid were found to be non-pathogenic on bean (*Phaseolus*

vulgaris) plants (Godoy *et al.*, 1990). *B. cinerea* has also been shown to produce oxalic acid both *in vitro* and *in planta*. A gene encoding oxaloacetate hydrolase, an enzyme which converts oxaloacetate into pyruvate and oxalate, has been cloned and characterised. Mutants of *BcoahA* are defective in oxalate production but retain the ability to produce sclerotia (Han *et al.*, 2007). The effect of this mutation on pathogenesis has not yet been investigated.

1.7.10 Phytohormones

Like plants, fungi also produce different phytohormones which may act as virulence factors during pathogenesis. Recent studies have shown that *B. cinerea* produces two phytohormones, ethylene (ET) (Chagué *et al.*, 2002) and abscisic acid (ABA) (Inomata *et al.*, 2004; Siewers *et al.*, 2004). The ET production pathway in *B. cinerea* was shown to be distinct from that in plants. In plants, ET is produced from methionine through the intermediates S-adenosyl methionine (SAM) and 1-aminocyclopropane-1-caboxylase (ACC) (Johnson and Ecker, 1998). In *B. cinerea*, production also starts from methionine through the intermediate α-keto-γ-methylbutyric acid (KMBA) (Chagué *et al.*, 2002). This intermediate accumulates in the dark and is photo-oxidized to ET in the presence of light. It is a spontaneous reaction and no enzyme activity is involved.

Chagué *et al.* (2006) recently studied ET sensing and signal transduction in *B. cinerea*. Mutants affected in the *BCG1* gene (Schulze Gronover *et al.*, 2001) showed ET insensitivity and *in vitro* ET overproduction. Macroarray analysis of cDNA from ET treated and starved *B. cinerea* mycelia revealed that ET activates a number of transcriptional changes. Among the ET-induced *B. cinerea* genes identified was a general stress response element (*Bchsp30*) and snod-prot-like gene (*Bcspl1*). Exogenous application of ET induced expression of *Bchsp30* in the wild-type and *bcg1* mutants after 24 and 48 hrs, however expression of *Bcspl1* at both time points was only detectable in the wild-type and not the *bcg1* mutants. This indicated that expression of *Bcspl1* but not *Bchsp30* was under the regulation of *Bcg1* which implicates other ET signalling pathways.

Blocking ET production in *Nicotiana benthamiana* plants with aminoethoxyvinylglycine (AVG) resulted in complete blockage of ET production as a result disease developed much faster in AVG treated compared to AVG untreated leaves. AVG inhibits the biosynthesis of ACC from SAM but because this pathway is only common to plants, this inhibition does not affect fungal ET biosynthesis. Inoculated AVG treated leaves developed disease similar to the control when placed under an ET atmosphere. High levels of ET were observed by 24 hrs after inoculation of infected non-AVG treated leaves and continued to increase reaching a plateau of 72 hrs after inoculation. Inoculating *N. benthamiana* leaves with *bcg1* mutants resulted in significant amounts of ET during the first 24 hrs after inoculation although levels dropped back to basal levels. Similar to the wild-type, no ET was could be detected in AVG-treated leaves. Consistent with these results *in planta* expression of *Bcspl1* was observed as early as 24 hrs and remained high during the first 72 hrs after inoculation. Expression of *Bcspl1* expression was delayed by 24 hrs in AVG treated leaves. These results indicated that plant ET induces the the expression of *Bcspl1* in the early stages of infection however, other signals might stimulate its expression during the later infection stages. The authors postulated that the *Bcspl1* gene product may act as an elicitor of defence responses during the early stages as its gene product may be recognized by the plant defence system resulting in reduced disease development in infected plants, however, it may also function as a virulence factor during the later stages of infection.

In plants, ABA regulates various physiological reactions such as induction of adaptive responses to water deficiency and low temperature in plants (Leung and Giraudat, 1998). ABA treatment was also shown to suppress phytoalexin synthesis as well as inhibition of the activity and transcript accumulation of phenylalanine ammonium lyase (PAL) (Mauch-Mani and Mauch, 2005). Mutants deficient in ABA such as *sitiens* of tomato display increased resistance to infection by *B. cinerea* (Audenaert *et al.*, 2002). Application of ABA restores susceptibility of *sitiens* but also increases susceptibility of wild-type tomato plants and cut rose flowers (Wedding *et al.*, 1996) to *B. cinerea*. Other phytopathogenic fungi such as various *Cercospora* species have also been shown to produce ABA (Kitagawa *et al.*, 1995). Kettner and Dörffling (1995) performed a detailed study of the role of ABA in the tomato-*B. cinerea* interaction and concluded that ABA biosynthesis by *B. cinerea* was

stimulated by the host plant. Based on the effects of ABA (Wedding *et al.*, 1996; Audenaert *et al.*, 2002), it is possible that *B. cinerea* utilizes ABA as a pathogenicity factor. ABA synthesized in *B. cinerea* is produced via the carotenoid pathway from farnesyl diphosphate (Inomata *et al.*, 2004). Four genes (*Bcaba1*, *Bcaba2*, *Bcaba3* and *Bcaba4*) involved in the ABA biosynthetic pathway have been identified (Siewers *et al.*, 2004, 2006). Targeted gene inactivation has provided evidence for the involvement of *Bcaba1*, *Bcaba2* and *Bcaba3* in ABA biosynthesis and suggested a contribution of *Bcaba4* (Siewers *et al.*, 2006). The molecular role of ABA in *B. cinerea* has not yet been determined.

B. cinerea is an ultimate necrotroph; this pathogen uses multiple strategies to achieve effective colonization of its hosts. For instance, it produces various pathogenicity factors aimed at destroying or manipulating host defence mechanisms. The process of infection of this pathogen seems to be more complex than had first been envisaged. Penetration of the host surface seems not to involve any enzymatic activity but a physical process dependent on mechanical forces. Some of the enzymes that were originally thought to have a significant role in pathogenesis seem to be utilized by the pathogen in the decomposition of plant biomass and eventual conversion to fungal biomass. Mutations in some of these genes can lead to strains that are resistant to various stresses an indication that the fungus can easily evolve to adapt to various environments hence its wide distribution.

1.8 Host resistance to *B. cinerea*

In the previous section, it has been demonstrated that *B. cinerea* employs a number of mechanisms to detect its potential hosts which is followed by deployment of various pathogenicity factors to achieve successful colonization. However, plants have also evolved mechanisms of protection against *B. cinerea* and other pathogens. They include those that are constantly present (constitutive or preformed) and those that are appropriately deployed in response to pathogen recognition (inducible).

1.8.1 Preformed defences

Preformed defences are the type of defences that are present within the plant even in the absence of infection. They offer the first line of defence that pathogens must overcome to cause infection (Mysore and Ryu, 2004). The cuticle is an example of this type of defence and its disruption through wounding or any other treatment results in more rapid infection by *B. cinerea* (Harrison, 1988). The thickness of the cuticle also plays an important role in impeding pathogen penetration; for example, in tomato and rose plants, resistance to *B. cinerea* was correlated with cuticle thickness (Rijkenberg *et al.*, 1980; Hammer and Evensen, 1994).

1.8.2 Inducible defences

1.8.2.1 Elicitors

When pathogens alight on their preferred host, they produce a number of pathogenicity factors whose main aim is either to manipulate or destroy host defence mechanisms. Such pathogenicity factors can function as elicitors of defence responses in plants. Elicitors fall into three categories; MAMPs (microbe-associated molecular patterns), effectors (*avr* proteins) and MIMPs (microbe-induced molecular patterns) (Mackey and McFall, 2006). MAMPs are small molecular motifs consistently found in all microbes. Examples include fungal cell wall components such as chitin and glucan and macromolecular structures such as flg22, a 22-amino acid peptide from the bacterial flagellin protein (Hahn, 1996; Nurnberger and Brunner, 2002; Montesano *et al.*, 2003). MAMPs are highly conserved and difficult for the pathogen to shed or alter. They are recognized by MAMP receptors within host plants if they are secreted or liberated from the pathogen by host enzymes or when the pathogen dies (Mackey and McFall, 2006). MAMPs are also referred to as PAMPs (pathogen-associated molecular patterns) however the term MAMP is inclusive of all molecules expressed by all microbes (pathogenic and non-pathogenic) that can activate immune responses in plants and animals (Jones and Dangl, 2006; Bent and Mackey, 2007).

Effectors are pathogen derived molecules whose inherent activity is to interact with and thus affect the host (Mackey and McFall, 2006). Examples include CWDEs (e.g. polygalacturonases), toxins (e.g. botrydial), phytohormones, proteases as well as many proteins of unknown function (Mackey and McFall, 2006). Unlike MAMPs, pathogens may delete their effectors or alter them through mutation, this is essential as hosts evolve to recognize them (Bent and Mackey, 2007). They can be recognized by specific plant proteins referred to as R proteins (Dangl and Jones, 2001; Ingle *et al.*, 2006). This recognition may either occur directly as in the case of AvrPto, an Avr protein of *Pseudomonas syringae pv tomato* which is recognized by Pto, an R protein of tomato (Shan *et al.*, 2000) or indirectly as in the case of AvrRpt2, of *Pseudomonas syringae* and RPS2 of *Arabidopsis* (Axtell *et al.*, 2003; Mackey *et al.*, 2003). A MIMP is a product that results from the virulence activity of an effector (Mackey and McFall, 2006). Examples of MIMPs include the enzymatic products of CWDE such as the oligogalacturonides (OGAs) (Hahn, 1996; Ridley *et al.*, 2001; Juge, 2006). These can also activate plant defence responses.

In biotrophic and hemibiotrophic interactions, specific *R* genes in the host recognize specific *avr* proteins in a gene-for-gene relationship (Hammond-Kosack and Jones, 1997). However, necrotrophs produce a variety of non-specific elicitors which are recognized by their potential receptors (Ferrari *et al.*, 2007). Some of these elicitors are MAMPs (chitin and glucans), others are effectors (polygalacturonases) (Poinssot *et al.*, 2003) while others are MIMPs (OGAs). The role of polygalacturonases in *B. cinerea* interactions has been elaborated. Many studies have demonstrated the role of these elicitors in *B. cinerea* interactions. For instance, Poinssot *et al.* (2003) demonstrated the ability of the *B. cinerea* polygalacturonase *BcPG1* to elicit defence responses in grapevine while Aziz *et al.* (2004) showed that exogenous treatment with OGAs protected grapevine (*V. vinifera*) leaves against *B. cinerea*. Ferrari *et al.* (2007) also recently demonstrated the role of OGAs in resistance of *Arabidopsis* to *B. cinerea*. Recognition of elicitors activates a multitude of defence responses which include production of signalling compounds such as AOS, salicylic acid (SA), jasmonic acid (JA), nitric oxide (NO) and ET. These in turn regulate further defence responses such as synthesis and accumulation of phytoalexins, synthesis of enzymes such as glycosyl hydrolases capable of attacking surface polymers of pathogens, strengthening of the cell through deposition of callose, hydroxyproline-

rich glycoproteins and lignin and hypersensitive response (Hahn, 1996; Wan *et al.*, 2002).

1.8.2.2 Polygalacturonase-inhibiting proteins

Plants have evolved mechanisms to counteract the degradation effects of polygalacturonases by expressing proteins known as polygalacturonase-inhibiting proteins (PGIPs) (De Lorenzo *et al.*, 2001). PGIPs are localized in the cell walls of many dicotyledonous plants and are effective against polygalacturonases released by fungi but not those of bacteria or plants and have no activity against other known CWDEs (Cervone *et al.*, 1990). They belong to the leucine-rich repeat (LRR) superfamily of proteins (Mattei *et al.*, 2001; Di Matteo *et al.*, 2003), a family shared by many other plant proteins involved in the recognition of pathogens, such as the majority of resistance gene products like *Xa21* of rice (Wang *et al.*, 1996) and receptors of MAMPs, such as FLS2 (Flagellin sensitive2) (Gómez-Gómez *et al.*, 2001). They are induced by various stimuli which include pathogen attack, wounding, elicitors such as the OGAs, cold treatment and signalling molecules (e.g. SA and JA) (De Lorenzo *et al.*, 2001; Ferrari *et al.*, 2003). Their role in defence against *B. cinerea* and other fungal pathogens has been elaborated in a number of studies in which they have been shown to retard polygalacturonase function, prevent cell wall degradation, and limit fungal growth and colonization. Overexpression of genes encoding these proteins in tobacco (*Nicotiana tabacum*) (Manfredini *et al.*, 2005), tomato (*Lycopersicon esculentum*) (Powell *et al.*, 2000), grapevine (*V. vinifera*) (Agüero *et al.*, 2005) and *Arabidopsis* (Ferrari *et al.*, 2003) was shown to limit colonization by *B. cinerea* which resulted in reduced disease symptoms.

1.8.2.3 Phytoalexins

Phytoalexins also play a significant role in defence against *B. cinerea* and other necrotrophs (Elad, 1997; Prins *et al.*, 2000b). They are produced in both resistant and susceptible plants, however, they generally accumulate more rapidly and to higher levels with positive results in resistant plants (Morrissey and Osbourn, 1999). For instance, accumulation of the stilbene phytoalexin resveratrol in grapevine and pisatin in pea plants was shown to correlate with increased resistance to *B. cinerea*

(Sbaghi *et al.*, 1995). Overexpression of grapevine stilbene synthase genes that encode enzymes involved in the biosynthesis of stilbene and resveratol resulted in increased resistance to *B. cinerea* in tobacco and tomato (Hain *et al.*, 1993; Thomzik *et al.*, 1997). In *Arabidopsis*, the role of the main phytoalexin camalexin in protection against *B. cinerea* is not very clear. In some studies, it has been shown that its accumulation does not affect growth of *B. cinerea* but inhibits growth of other pathogens such as *A. brassicicola* and *Erysiphe orontii* (Thomma *et al.*, 1999; Govrin and Levine, 2002) while in another study, it was shown that camalexin is necessary for local resistance to *B. cinerea* (Ferrari *et al.*, 2003). However, Kliebenstein *et al.* (2005) recently demonstrated that camalexin may offer protection against some but not all *B. cinerea* isolates. In their study, they tested three isolates of which one displayed *in vitro* and *in planta* camalexin tolerance during spore germination and hyphal growth but the other two displayed camalexin sensitivity. These two isolates produced larger lesions on camalexin deficient *Arabidopsis* mutants than the wild-type an indiction that camalexin impeded their development. Because one of the isolates displayed *in vitro* camalexin tolerance, it is possible that some *B. cinerea* isolates have developed mechanisms for detoxification of this phytoalexin as in the case of resveratrol (Schouten *et al.*, 2002).

1.8.2.4 Signalling pathways

SA-dependent signalling pathway

The role SA plays both in the establishment of systemic acquired resistance (SAR), and elaboration of local defence responses has been well elucidated in many studies (Feys and Parker, 2000; Kunkel and Brooks, 2002; Durrant and Dong, 2004; Glazebrook, 2005). Endogenous levels of this signalling compound not only increase in pathogen challenged tissues but also correlate with elevated expression of genes encoding pathogenesis-related (PR) proteins especially *PR1*, *PR2* (β-1,3-glucanase) and *PR5* (osmotin) (Glazebrook, 2001; Kunkel and Brooks, 2002). Exogenous application of SA or its functionally related analogues, 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) to wild-type plants also enhances host resistance to a broad range of pathogens in addition to expression of *PR* genes (Glazebrook, 2001). SA is synthesized through the phenylpropanoid pathway either from phenylalanine

in a reaction catalyzed by phenylalanine ammonia lyase (PAL) (Lee *et al.*, 1995) or directly from chorismate in a reaction catalyzed by isochorismate synthase (ICS) and isochorismate pyruvate lyase (IPL) (Serino *et al.*, 1995). However, Ferrari and associates (2003) reported that the IPL pathway is more important in resistance against *B. cinerea*.

Regulation of mechanisms that lead to successful defence against biotrophic pathogens (e.g. *E. orontii*, *Peronospora (Hyaloperonospora) parasitica*, and *Pseudomonas syringae*) is known to be mediated by SA (Cao *et al.*, 1994, 1997; Delaney *et al.*, 1994) however, its role in defence against *B. cinerea* and other necrotrophic pathogens seems to depend on host plants (Achuo *et al.*, 2004). For instance, foliar application of SA enhanced resistance to *B. cinerea* in bean plants (De Meyer *et al.*, 1999) while BTH reduced disease development in tomato plants (Audenaert *et al.*, 2002: Achuo *et al.*, 2004) and poinsettia leaves (Kulek and Floryszak-Wieczorek, 2002). A soil drench application of BTH one day prior to inoculation also markedly reduced infection of *Arabidopsis* by two *B. cinerea* strains (Zimmerli *et al.*, 2001). However, Govrin and Levine (2002) reported no effect of exogenous SA and BTH on resistance of *Arabidopsis* to *B. cinerea*.

In tobacco, soil treatment with BTH did not suppress *B. cinerea* (Achuo *et al.*, 2004) while foliar sprays with the same compound had no effect on resistance of tobacco to *B. cinerea* (Friedrich *et al.*, 1996; Achuo *et al.*, 2004) and another necrotroph *Alternaria alternata* (Friedrich *et al.*, 1996). But Chivasa *et al.* (1997) and Murphy *et al.* (2000) reported a delay in the development of *B. cinerea* after foliar application of SA on tobacco plants. It is surprising that application of SA induced resistance of tobacco to *B. cinerea* yet BTH had no effect on the same interaction. The authors attributed this discrepancy in results to the type of plants and method of inoculation used in the two studies. In the study by Chivasa *et al.* (1997) and Murphy *et al.* (2000), tobacco plants as young as three weeks were used and were watered with 1 mM SA everyday for 5 days while in the study by Achuo *et al.* (2004), only two soil applications of BTH were made at planting and at 5 days before inoculations. Only one foliar application with a 10 mg a.i. L^{-1} solution was made in this study.

Similar observations as those made with SA and BTH have been recorded in transgenic plants engineered to produce *NahG*. *NahG* is an SA metabolizing enzyme from *Pseudomonas putida* that converts SA into catechol. These plants exhibit reduced levels of SA and even though they develop an HR following challenge by an avirulent pathogen, they do not exhibit systemic expression of *PR1*, *PR2* and *PR5*. In addition, local expression of PR proteins is reduced. They are also susceptible to biotrophic pathogens such as the bacterium *P. syringae*, the oomycete *H. parasitica* and fail to develop resistance to subsequent pathogen attacks (Delaney *et al.*, 1994; Lawton *et al.*, 1995). Increased susceptibility of *NahG Arabidopsis* plants to infection by *B. cinerea* compared to the control plants was recently reported in three different studies (Zimmerli *et al.*, 2001; Govrin and Levine, 2002; Ferrari *et al.*, 2003) suggesting a role for SA in basal resistance to *B. cinerea* in *Arabidopsis*, however, Thomma *et al.* (1998) observed no difference in infection levels between the mutants and wild-type plants.

Govrin and Levine (2002) attributed the differences between their results and those of Thomma *et al.* (1998) to differences in virulence of the *B. cinerea* strains tested. Because Zimmerli *et al.* (2001) included the strain tested by Thomma *et al.* (1998) in their work, they attributed the differences to inoculation conditions. Ferrari *et al.* (2003) also demonstrated that accumulation of catechol was not responsible for the enhanced susceptibility of *NahG* plants since exogenous catechol enhanced rather than decreased resistance to *B. cinerea*. In tomato, *NahG* leaves were also slightly more susceptible to *B. cinerea* than the wild-type (Audenaert *et al.*, 2002; Achuo *et al.*, 2004) while in tobacco, *NahG* plants displayed similar levels of susceptibility to *B. cinerea* as wild-type suggesting that endogenous SA does not play a role in this interaction.

In *Arabidopsis*, it was recently demonstrated that SA synthesized via the PAL and not the ICS1 pathway mediates local resistance to *B. cinerea* and requires *EDS4* and *PAD2* but not *SID2*, *EDS5* or *PAD4* (Ferrari *et al.*, 2003). However, *EDS5* and *PAD4* were shown to be necessary for the restoration of resistance to *B. cinerea* in *Arabidopsis ssi2* mutant plants which exhibit high susceptibility to this pathogen (Nandi *et al.*, 2005). The *Arabidopsis SSI2* protein is involved in lipid metabolism while *PAD4* exhibits homology to lipases and esterases (Jirage *et al.*,

1999). Another gene shown to be important in resistance against *B. cinerea* but dependent on normal levels of SA is the plasma membrane-localized serine/threonine protein kinase, *BIK1* (*BOTRYTIS INDUCED KINASE1*). The *bik1* mutant plants are more susceptible to *B. cinerea* and *A. brassicicola* than the wild-type and show attenuated expression of *PDF1.2* (Veronese *et al.*, 2006). They also accumulate increased levels of SA before and after infection with *B. cinerea* suggesting that *BIK1* acts upstream of SA accumulation. It is possible that the wild-type *BIK1* acts by suppressing the SA pathway which increases JA signalling as the two pathways antagonize each other (Kunkel and Brooks, 2002). *BIK1* removal may enhance the SA pathway, which suppresses JA signalling hence the increased susceptibility to *B. cinerea* in *bik1* mutants.

The tomato ABA-deficient mutant *sitiens* displays increased resistance to *B. cinerea* that is dependent on SA signalling (Audenaert *et al.*, 2002). The effect of ABA was demonstrated by applying ABA which resulted in enhanced susceptibility to *B. cinerea* in both the mutants and wild-type plants. *Sitiens* mutants also displayed higher levels of PAL activity than the wild-type an indication that PAL activity in wild-type plants was partially repressed by ABA. However, a recent study by Asselbergh *et al.* (2007) demonstrated that resistance in *sitiens* was also due to its ability to accumulate H_2O_2 in the leaf epidermal cell walls during the early hours of the interaction. The role of H_2O_2 in this interaction was demonstrated by application of antioxidants (e.g. catalase and ascorbate) and inhibition of AOS generation with diphenilene iodonium (DPI) which resulted in a significant increase in susceptibility. Although *B. cinerea* has been shown to utilize AOS to colonize host tissue, AOS accumulate very early in *sitiens* leading to fast induction of defence responses. This offers efficient protection to these mutants against *B. cinerea*.

SA dependent responses have also been shown to mediate β-aminobutyric acid (BABA) mediated protection of *Arabidopis* against *B. cinerea* (Zimmerli *et al.*, 2001). The involvement of SA, JA or ET in BABA-mediated resistance in this interaction was demonstrated in transgenic *Arabidopsis* mutants affected in the three signalling pathways. BABA protected mutants defective in the JA (*coi1*, *coronatine insensitive 1*) and ET (*etr1*, *ET triple response 1*) pathways but not those impaired in the SA pathway (*NahG* and *npr1*, *non-expressor of PR1*). Treatment

with BABA also potentiated the plant to rapidly and intensively express the SA-dependent *PR1* rather than the JA/ET-dependent *PDF1.2*. BABA-induced resistance in other necrotrophic interactions such as *A. brassicicola* and *P. cucumerina* in *Arabidopsis* was attributed to ABA-dependent priming (Ton and Mauch-Mani, 2004) while induced resistance to *Alternaria brassicae* in *Brassica juncea* was attributed to enhanced expression of *PR* genes independent of SA and JA accumulation (Kamble and Bhargava, 2007).

JA-dependent signalling pathway

JA is a 12-carbon fatty acid derivative that is synthesized via the octadecanoic pathway from linolenic acid (Creelman and Mullet, 1997; Wasternack and Parthier, 1997). It is an important regulator of many developmental processes in plants which include root growth, fruit ripening, senescence, pollen development, tuber formation, and tendril coiling (Creelman and Mullet, 1995; Wasternack and Parthier, 1997; Reymond and Farmer, 1998; Weber, 2002). JA and methyl jasmonate (MeJA) have been shown to play a central signalling role in plant defence responses against insect attack and infection by necrotrophic pathogens (Xie *et al.*, 1998). Along with ET, JA mediates the activation of induced systemic resistance (ISR), an induced resistance response associated with the root colonization by nonpathogenic Rhizobacteria (Pieterse *et al.*, 2001b). In *Arabidopsis*, JA mechanisms modulate local and systemic resistance to *B. cinerea*. Exogenous application of JA or MeJA leads to expression of the *Arabidopsis PDF1.2* and *THI2.1* genes both of which are also induced following infection by *B. cinerea* and other necrotrophic pathogens (Penninckx *et al.*, 1998; Diaz *et al.*, 2002). These genes which are induced locally and systemically are used as markers for the JA (*THI2.1*) and JA/ET (*PDF1.2*) signalling pathways. *PDF1.2* and *THI2.1* are not induced in response to SA and can be expressed in *NahG* plants.

Mutations impairing JA biosynthesis (e.g. *Arabidopsis fad 3,8,7* triple mutant (*fatty acid desaturase 3,8,7*) and tomato *def1* (*defenseless 1*)) or perception (e.g. *Arabidopsis coi1* and *jar1* (*jasmonate resistant 1*) mutations) enhance susceptibility of the affected plants to *B. cinerea* in addition to other necrotrophic pathogens (e.g. *A. brassicicola*, *Pythium*, and *Erwinia carotovora*) (Ferrari *et al.*,

2003; Thomma *et al.*, 1999). A number of mutations in other genes such as *BOS1* (*BOTRYTIS SUSCEPTIBLE 1*), *BOS2*, *BOS3*, and *BOS4* (Mengiste *et al.*, 2003; Veronese *et al.*, 2004), *ESA1* Tierens *et al.* (2002) and *iop1* (Penninckx *et al.*, 2003) reduce levels of *PDF1.2* expression and display increased susceptibility of *Arabidopsis* to *B. cinerea*. Excluding *BOS2*, all other mutations led to increased susceptibility to other necrotrophs such as *A. brassicicola* (*bos1*, *bos3*, *bos4*, *esa1* and *iop1*) and *Plectosphaerella cucumerina* (*esa1* and *iop1*). All mutants retained wild-type levels of resistance to biotrophic pathogens except *bos4*. Unlike *BOS1* which is now known to encode an R2R3MYB transcription factor protein (Mengiste *et al.*, 2003), *BOS2*, *BOS3*, *BOS4*, *ESA1*, and *IOP1* have not yet been cloned.

ET-dependent signalling pathway

ET is a key regulator of many plant processes such as seed germination, seedling growth, cell expansion, flower development, leaf and petal abscission, root nodulation, organ senescence (Mattoo and Suttle, 1991; Johnson and Ecker, 1998; Bleecker and Kende, 2000; Binder *et al.*, 2004; Guo and Ecker, 2004; Chen *et al.*, 2005) and resistance to both biotic and abiotic stresses (Roman *et al.*, 1995; O'Donnell *et al.*, 1996; Penninckx *et al.*, 1998). In plants, ET is synthesized from SAM to ACC which is subsequently converted to ET in reactions catalyzed by ACC synthase (ACS) and ACC oxidase respectively (Ecker, 1995; Chae *et al.*, 2003; Chae and Kieber, 2005). Production of ET may be induced by pathogen invasion and elicitors (Mauch *et al.*, 1984; Penninckx *et al.*, 1998). Its production leads to activation of a number of plant defence responses such as production of phytoalexins, PR proteins (e.g. *PDF1.1* and *PDF1.2*), induction of the phenylpropanoid pathway and cell wall alterations (Mauch *et al.*, 1984; Ecker and Davis, 1987).

In *Arabidopsis*, ET plays a pivotal role in the defence against *B. cinerea* and other necrotrophic pathogens. *Arabidopsis* mutants (*ein2*) which are impaired in its perception display enhanced susceptibility to *B. cinerea* (Thomma *et al.*, 1998, 1999). The *EIN2* gene encodes a membrane-associated signal transduction component, its amino terminus shows homology to the Nramp family of metal ion transporters (Alonso *et al.*, 1999). Overexpressing the *ETHYLENE RESPONSE FACTOR1* (*ERF1*) enhances *Arabidopsis* resistance to *B. cinerea* and another

necrotroph *P. cucumerina* (Berrocal-Lobo *et al.*, 2002). *ERF1* is a down stream component of the ET signalling pathway (Solano *et al.*, 1998).

ET has also been shown to play an important role in protection of tomato plants against infection by *B. cinerea* (Diaz *et al.*, 2002). Pretreatment of wild-type and *Never ripe* mutants resulted in reduced susceptibility to *B. cinerea*. Treatment with ET perception inhibitors reduced the proportion of plants infected by *B. cinerea*. The role of ET in this interaction was further demonstrated in two genotypes, *epinasitic* mutants which are constitutively activated in a set of ET responses and a transgenic line (accession no. UC8338) producing negligible amounts of ET. *Epinasitic* mutants displayed a significant reduction in the percentage of expanding lesions as compared with the wild-type while UC8338 was more susceptible to *B. cinerea* infection than its non-transgenic progenitor. In this study they also demonstrated that JA and wounding act independently of ET to confer resistance in tomato to *B. cinerea*.

Cross-talk between signalling pathways

Enormous evidence has demonstrated that the three pathways do not function in isolation but are involved in complex signalling networks in which each pathway may have a positive or negative influence on another pathway (Kunkel and Brooks, 2002). Interactions between the SA and JA signalling pathways seem to be antagonistic in most cases (Kunkel and Brooks, 2002). For example, SA inhibits synthesis of JA and prevents the accumulation of proteinase inhibitors in response to JA (Pena-Cortés *et al.*, 1993; Doares *et al.*, 1995). In addition to its functional analogs (BTH and INA), SA has also been shown to prevent the expression of JA-dependent defence genes (Gupta *et al.*, 2000). Negative crosstalk between JA and SA seems to be mediated by the MAP KINASE 4 (MPK4) and involves *EDS1* (*ENHANCED DISEASE SUSCEPTIBILITY 1*) and *PAD4* (*PHYTOALEXIN DEFICIENT 4*) (Petersen *et al.*, 2000; Brodersen *et al.*, 2006). *EDS1* and *PAD4* affect this antagonism by activating SA-dependent defences and repressing JA/ET-dependent defences. Both these functions are regulated by MPK4.

It appears *B. cinerea* capitalizes on this antagonism to promote host colonization. For example, *EDS1* and another gene *SGT1* were activated by *B. cinerea* to enhance disease development in *N. benthamiana* (El Oirdi and Bouarab, 2007). Virus-induced gene silencing of both these genes enhanced resistance of *N. benthamiana* to *B. cinerea* an indication that the two genes are essential in colonization of the host by the pathogen. A node of convergence between SA and JA signalling seems to be the plant-specific transcription factor WRKY70 (Li *et al.*, 2004). Plants overexpressing WRKY70 showed decreased JA- but enhanced SA-dependent defence activation, hence improving resistance to *E. carotovora* and the biotroph *P. syringae* (Li *et al.*, 2004) an indication that WRKY70 integrates defence signals and therefore affects pathway activation. Not much evidence about interactions between SA and ET is available, however, Murray *et al.* (2005) recently demonstrated that resistance to *B. cinerea* in the *cir3* mutant is dependent on functional ET and SA signalling pathways. This mutant displays increased resistance to *B. cinerea*.

A number of studies have demonstrated that the JA and ET signalling pathways positively interact with each to induce a number of defence-related genes. For instance, exogenous application of JA and ET results in expression of *PDF1.2*, *HEL*, and *CHIB* in *Arabidopsis* and *osmotin* and *PR1b* in tobacco (Kunkel and Brooks, 2002). The genes *PDF1.2* and *HEL* are well known to be induced in *Arabidopsis* in response to *B. cinerea* and other necrotrophs. In a microarray study, Schenk *et al.* (2000) also demonstrated that 50% of the genes induced by ET were also induced by MeJA. Some of the genes induced by the two compounds were also induced in response to *A. brassicicola*. The positive cooperation of the JA and ET signalling pathways is also demonstrated in activation of *ERF1*. The induction of *ERF1* by JA is dependent on *EIN2* while its induction by ET is dependent on *COI1* (Lorenzo *et al.*, 2003). Constitutive expression of *ERF1* has been shown to enhance resistance to *B. cinerea* (Berrocal-Lobo *et al.*, 2002).

Despite the multitude of defence mechanisms, many plants still succumb to *B. cinerea*. It is therefore necessary that appropriate management practices are employed so as to keep this pathogen and its effects under control. Unlike other fungal pathogens, it is not easy to achieve a satisfactory level of control of *B. cinerea*. This is attributed to a number of reasons; first, *B. cinerea* uses various modes to

attack its hosts; second, the wide range implies inoculum is always available from various sources; third, it can survive for short periods as mycelia and/or conidia or for extended periods as sclerotia in crop debris and lastly it can infect crops at any stage of growth and any part of the plant (Williamson *et al.*, 2007).

1.9 Management

Because of the complexity of this pathogens, successful management can not be achieved by using one strategy but many strategies based on an integrated approach. For this to be achieved, a more detailed understanding of the host-pathogen interaction, the micro-environment in which the fungus operates and the microbial competitors on the host is essential (Williamson *et al.*, 2007). Fortunately most of this information is readily available for *B. cinerea*. Among the strategies tested and found to be efficient at containing this fungus are cultural, biological and chemical control measures.

1.9.1 Cultural control

Cultural control measures are aimed at reducing the source of inoculum and providing conditions that may reduce development and spread of the pathogen. Such measures include ensuring good sanitation practices such as collecting and removing infected plant debris from the field and stored produce. This helps reduce conidia and vegetative hyphae which may serve as sources of inoculum (Hausbeck and Moorman, 1996). Pruning crops to remove stressed and aging parts is also effective as these can be used opportunistically to gain entry into the plant. In addition, removal of these stressed and aging parts helps deny *B. cinerea* ground for sporulation. In studies by Hausbeck and Pennypacker (1991) they found that an increase in wounded and senescent tissue was associated with increased atmospheric conidial concentrations. They also showed that the major source of inoculum for infecting geranium stems during harvesting of cuttings was sporulating *B. cinerea* on necrotic leaves at the base of stock plants. Similar observations on conidial concentrations were made by Kerssies (1993). He showed that the number of airborne conidia of *B. cinerea* increased with the amount of dead gerbera tissue as the crop aged. For

similar reasons, fallen plant parts such as flowers, leaves and those that remain after harvest, should be immediately removed (Pappas, 2000).

High humidity, reduced light and moderate temperatures offer the best conditions for development of *B. cinerea*. Crop management practices that can create an open canopy so as to provide adequate air movement and good light interception will reduce the rate of progress of the disease (Williamson *et al.*, 2007). Adequate air circulation created by an open canopy helps dry water drops from rain or irrigation and reduce relative humidity, both of which are very essential for conidial germination. In greenhouses, arranging plants in rows parallel to the air flow with space between rows helps reduce relative humidity (Trolinger and Strider, 1984). Creating adequate but optimal spacing between plants creates a less dense plant canopy and allows better light penetration which not only reduces relative humidity but also the number of senescing tissue such as leaves of the lower plant parts. This removes potential infection and sporulating sites for *B. cinerea* (Hausbeck and Moorman, 1996).

Excessive use of nitrogen fertilizers should be avoided as it encourages not only rapid vegetative growth but also increases the risk of grey mold and other diseases (Williamson *et al.*, 2007). However, application of calcium containing fertilizers such as calcium nitrate resulted in the reduction of ghost spot of tomato (Elad and Volpin, 1993). This increased resistance in tomato plants after application of calcium fertilizers was attributed to 1) the strengthening effect of membranes by calcium ions which reduced leakage of nutrients from tissues and 2) reduction in ethylene production. Calcium sulphate and calcium dihydrogen phosphate can be used as replacements for calcium nitrate. As mentioned in previous sections, *B. cinerea* is as important on post-harvest produce as it is on plants before harvest (Pappas, 2000). Proper management of *B. cinerea* at post-harvest level is very important as it is not possible to apply chemicals. Practices that promote proper aeration such as adequate ventilation should be adhered to in storage compartments. Harvested produce should be kept under low temperatures during storage and transportation (Williamson *et al.*, 2007). It is also important to examine produce and remove all infected candidates as these will be the sources of inoculum.

1.9.2 Biological control

Biological control of plant diseases can be defined as the decrease of inoculum or the disease producing activity of a pathogen accomplished through one or more organisms including the host plant but excluding man (Baker, 1987). Many organisms which are in nature nonpathogenic to the host in question, have been shown to suppress the growth of pathogens that affect that host. They achieve this through many ways which include competition for nutrients, production of inhibitory metabolites and/or parasitism (Elmer and Reglinski, 2006). In the case of *B. cinerea*, a lot of studies have been done resulting in the discovery of a number of microorganisms that naturally suppress *B. cinerea*. Examples of these microorganisms include yeast and yeast-like organisms (e.g. *Aurobasidium pullulans*, *Candida guilliermondii*, *Candida oleophila*, *Candida pulcherrina*, *Candida sake* and *Trichosporon pullulans* (Dik *et al.*, 1999; Saligkarias *et al.*, 2002; Elmer and Reglinski, 2006)); filamentous fungi (e.g. *Galactomyces geotrichum*, *Gliocladium roseum*, *Trichoderma* spp, and *Ulocladium atrum* (Köhl *et al.*, 1998; Hjeljord and Tronsmo, 2003; Elmer and Reglinski, 2006)) and bacteria (e.g. *Bacillus circulans*, *Bacillus subtilis*, *Brevibacillus brevis*, *Pseudomonas fluorescens*, *Serratia liquefaciens*, and *Serratia marcescens* (Leifert *et al.*, 1993; Iyozumi *et al.*, 1996; Elmer and Reglinski, 2006)).

Spraying crops with spore suspensions of the above organisms has been shown to significantly reduce disease incidence and severity of *B. cinerea* (Köhl *et al.*, 1998; Dik *et al.*, 1999; Saligkarias *et al.*, 2002). Several biological control products developed from some of these organisms have been approved for use on food and non-food plants in some countries (Williamson *et al.*, 2007). They are commercially produced and marketed under specific trade names. Examples include *Trichoderma harzianum* isolate T39 (Trichodex 20SP), (Elad, 2000). Trichodex 20SP has also been shown to be effective against *Pseuperonospora cubensis*, *S. sclerotiorum* and *Sphaerotheca fusca* (Elad, 2000). Others are *Trichoderma atroviride* (Sentinel) (www.agrimm.co.nz), *Ulocladium oudemansii* (Botry-Zen) (http://www.botryzen.co.nz), *Metschnikowia fructicola* (Shemer) (http://agrogreen.co.il), and *Bacillus subtilis* (Serenade) (http://www.serenadegarden.com).

Although not strictly biological control, volatile compounds (volatiles) produced by plants are also able to effectively reduce disease incidence and severity. Volatiles are defined as those compounds that have a relatively high vapour pressure at physiological temperatures hence are capable of approaching an organism in both the liquid and gas phase (Kulakiotu *et al.*, 2004). Plants produce a wide array of these compounds when stressed by various abiotic and biotic factors (Hatanaka. 1993). Those known to be produced include jasmonates, salicylates, green leafy volatiles and isoprenoids (Hatanaka, 1993; Pichersky and Gershenzon, 2002). Examples of green leafy volatiles include (E)-2-hexenal, (Z)-3-hexenal and n-hexanal (Pichersky and Gershenzon, 2002). Archbold *et al.* (1997) evaluated a number of these naturally occurring volatile compounds for their potential as biological control agents for *B. cinerea*. They found that some of these compounds either had inhibitory (fungistatic), lethal (fungicidal) or both effects. Among the volatile compounds they tested, hexanal, 1-hexanol, (E)-2-hexen-1-ol, (Z)-6-nonenal, (E)-3-nonen-2-one, methyl salicylate, and methyl benzoate exhibited fumigant properties for control of *B. cinerea* on blackberry, grape and strawberry. Other natural volatiles that have been assessed in different experiments include acetaldehyde, benzaldehyde, butanal, methyl salicylate, pentane and propanal (Wilson *et al.*, 1987; Hamilton-Kemp *et al.*, 1992).

Recently Kulakiotu *et al.* (2004) tested the effect of volatile compounds produced by the resistant grape cultivar Isabella (*Vitis labrusca L.*) on growth of *B. cinerea*. In addition to Isabella, they also included a susceptible cultivar Roditis (*V. vinifera L.*) for comparison. *In vitro* experiments showed that *B. cinerea* cultures grown in the presence of Isabella collapsed within 6 days and no conidia or sclerotia were formed. In contrast, sporulation and sclerotia formation were observed when *B. cinerea* was grown with Roditis. Besides the lack of sporulation, *in situ* experiments showed that Isabella was not infected by *B. cinerea* at all temperatures while fungal hyphae obtained from Roditis looked healthy and possessed abundant conidia. In the presence of Isabella volatiles, the incidence of infection of Roditis was significantly lower as compared to when Isabella volatiles were not used. Taken together, these results showed Isabella volatiles were highly effective in suppressing *B. cinerea* under conditions optimal for *B. cinerea* development and hence could be used as biological control agents against this pathogen.

1.9.3 Chemical control

Fungicides can be applied on a protective basis before disease develops, especially during periods of high humidity and cool temperatures (Hausbeck and Moorman, 1996). Examples of fungicides used over the years to control *B. cinerea* include the benzimidazoles (e.g. benomyl, thiabendazole, thiophanate-methyl and carbendazin); dicarboximides (e.g. chlozolinate, dichoran, iprodione, procymidone and vinclozolin): and multi-site toxicants (e.g. thiram, mancozeb, captan, dichlofluanid and tolylfluanid). Benzimidazoles act by inhibiting fungal growth through interfering with cell division. Dicarboximides show activity to both conidia and mycelium by affecting sensitivity to osmotic stress (Williamson *et al.*, 2007). Since the mid 1990s, new groups of fungicides that are effective against *B. cinerea* have been introduced. Examples include anilinopyrimidines (e.g. pyrimethanil, cyprodinil and mepanipyrim); phenylpyrroles (e.g. fludioxonil); and hydroxyanilides (e.g. fenhexamid). The three types of anilinopyrimidines have a site-specific mode of action, which involves interference with the biosynthesis of the amino acid methionine (Masner *et al.*, 1994), and the inhibition of secretion of hydrolytic enzymes (Miura *et al.*, 1994). Fludioxonil induces morphological alterations of the germ tubes which include swelling, abnormal branching and cell bursting. Fenhexamid has strong inhibitory effect on developmental stages that follow conidial germination such as germ tube elongation.

The disadvantage with using fungicides is the development of resistance in pathogen populations which is also true for *B. cinerea* (Latorre *et al.*, 2002; Sergeeva *et al.*, 2002; Leroux *et al.*, 2002; Leroux, 2004). Resistance to benomyl one of the benzimidazoles and cross-resistance to other benzimidazole fungicides as well as multiple resistance to both benzimidazole and dicarboximide fungicides has been reported (Moorman and Lease, 1992). Unlike dicarboximide-resistant strains which are less fit to survive than sensitive strains, benzimidazole-resistant strains are fit and competitive in nature even without selection pressure. This proposition is supported by studies which showed that benomyl-resistant strains persisted in the greenhouse for a long time after benomyl use had ceased (Leroux and Clerjeau, 1985; Georgopoulos and Skylakakis, 1986; Löcher *et al.*, 1987). Some captan-resistant isolates have also been found to have cross-resistance to various dithiocarbamates

(Leroux, 2004). *In vitro* and *in vivo* resistance to anilinopyrimidines by *B. cinerea* as well as cross-resistance between the three anilinopyrimidine fungicides has also been demonstrated (Forster and Staub, 1996).

A number of studies have been carried out to elucidate the genetic basis of resistance to benzimidazole and dicarboximide fungicides. Yarden and Katan (1993) observed three single-base pair mutations in the β-tubulin gene of the benomyl-resistant strains they tested. In the first case, codon 198, which encodes glutamic acid in the wild-type, was replaced by the alanine codon in strains highly resistant to benomyl but sensitive to *N*-phenylcarbamate (NPC) or the lysine codon in strains highly resistant to benomyl and resistant to NPC. The other mutation was at codon 200 encoding phenylalanine in the wild-type which was replaced by the tyrosine codon in strains moderately resistant to benomyl and resistant to NPC. Resistance to the dicarboximides was identified as a single polymorphic gene *Daf1* (Faretra and Pollastro, 1991) while the genes *Dic1* and *Dic2* were found to confer limited resistance to the multicite toxicant dichlofluanid (Pollastro *et al.*, 1996). The *Daf1* locus corresponds to *Bos1* a gene shown to encode an osmosensing histidine kinase (Cui *et al.*, 2002). This gene has been recently shown to mediate resistance to the phenylpyrroles and aromatic hydrocarbons in addition to the dicarboximides (Viaud *et al.*, 2006).

Continued development of resistance to fungicides by *B. cinerea* may also be attributed to the action of ABC transporter proteins. Schoonbeek *et al.* (2001) showed that the *B. cinerea* genome has genes that encode such proteins. Transcript levels of one of these genes, *BcatrB*, increased in the presence of phenylpyrrole fungicides, but not dicarboximides, anilinopyrimidines and lanosterol 14α-demethylase inhibitors. However, Hayashi *et al.* (2001, 2002) showed that another ABC transporter encoding gene *BcatrD* was induced by dicarboximides, anilinopyrimidines and lanosterol 14α-demethylase inhibitors. This implies *B. cinerea* has the ability to gain resistance to a combination of fungicides, a phenomenon referred to as multi-drug resistance. Even if new fungicide formulations are introduced, there will always be a possibility of development of fungicide resistant strains among the *B. cinerea* populations because of its versatility in defence. Strategies that can reduce the rate of appearance and establishment of strains resistant to fungicides should

be put in place and strictly adhered to by farmers. These strategies include the alternate application of fungicides with different modes of action and application of different fungicide combinations (Elad and Evensen, 1995; Melchers and Stuiver, 2000).

1.9.4 Breeding for resistance

All *B. cinerea* management strategies that have been discussed have advantages as well as shortcomings. Cultural control measures for instance are safe to the environment, farmer and consumer. However, they may be better suited for restricted environments such as those in greenhouses. This is because in open environments, inoculum is transferred long distances by air currents hence minimizing its sources in open fields may not necessarily deter *B. cinerea* epidemic development. Secondly, conditions that lead to the development of epidemics can be reduced in restricted environments whereas in open fields they are determined by changes in the macro-environment. Like cultural control, biological control is also safer as it does not present any risks associated with occupational exposure of workers to fungicides, consumers to fungicide residues in harvested crops and environmental degradation to nature (Utkhede and Mathur, 2002).

Biological control agents face a number of problems. For example, they often have restricted ranges of temperature or humidity for maximum microbial action, are influenced by fluctuations in natural populations of phylloplane microbes, are inefficient, lack persistence, and have non-target and human food safety effects (Williamson *et al.*, 2007). Greenhouses are more suited to this kind of strategy than open fields since environmental conditions can be well controlled (Paulitz and Belanger, 2001). But *B. cinerea* does not only affect crops grown in greenhouses, but also those in open fields. Therefore control strategies should be diverse in action targeting all possible environments. An example of such as a strategy would be application of fungicides.

Fungicides have been used for a long time with good results however there are limitations for using them as well. Development of fungicide resistant strains in pathogen populations over time renders them ineffective (Latorre *et al.*, 2002; Sergeeva *et al.*, 2002; Leroux *et al.*, 2002; Leroux, 2004). They can not be applied

at harvest time yet in *B. cinerea* epidemics this is the stage when the plant is more susceptible to infection (Murphy *et al.*, 2000). There is also a growing concern about the environment because of the continued use of chemicals in agriculture (Janisiewicz and Korsten, 2002; Spadoro and Gullino, 2005). Lastly, use of chemicals increases production costs and hence decimates even the little profits that can be realized from many farming enterprizes; besides they are only affordable by farmers in the developed World but not those from cash strapped nations like those in sub-Saharan Africa (Melchers and Stuiver, 2000). Hence development of cultivars resistant to *B. cinerea* remains the most cost effective approach in agriculture. For this to be done, it is necessary to first identify natural defences that protect plants from this pathogen. These can then be introgressed into economically important but highly susceptible crops (Elad and Evensen, 1995).

Necrotrophs such as *B. cinerea*, affect a variety of plant species hence resistance mediated in an *R-avr* relationship is unlikely to happen and gene-for-gene resistance has not been reported (Glazebrook, 2005). However, resistance against these pathogens is influenced by many genes with relatively smaller effects but which often act together. This line of belief was supported by Govrin and Levine (2002) who demonstrated that *B. cinerea* induces multiple defence responses in *Arabidopsis thaliana*. In their study with another necrotroph *A. brassicicola* Schenk *et al.* (2003) also reported the involvement of many genes in resistance to this necrotroph. Because disease resistance is a known quantitative trait as shown by these and many other studies, it is possible that there are regions on DNA that are associated with the resistance phenotype and possibly many genes underlie these regions. The best way to identify these regions is by carrying out quantitative trait loci (QTL) mapping studies. Besides identifying the loci that are responsible for the variation in complex quantitative traits, QTL mapping studies can also be used to determine the number, location and interaction of these loci and/or identify the actual genes that underlie these loci and their possible functions (Borevitz and Chory, 2004). Denby *et al.* (2004) performed a QTL mapping study between *B. cinerea* and *A. thaliana* and reported the presence of multiple small-to-medium effect QTLs governing susceptibility to this pathogen. Recently, partial resistance to *B. cinerea* has also been identified in several wild relatives of tomato (*Solanum lycopersicum*) such as *S. chilense*, *S. habrochaites* and *S. neorickii* (ten Have *et al.*, 2007).

In order to identify the loci involved in this resistance, an F_2 segregating population obtained from a cross between *S. habrochaites* accession LYC4 and *S. lycopersicum* accession moneymaker was used (Finkers *et al.*, 2007a). Three QTLs *Rbcq1*, *Rbcq2* and *Rbcq4* were identified. The abbreviation *Rbcq1* refers to "Resistance to *B. cinerea* QTL" and the number refers to the chromosome on which the QTL was identified and in this case chromosome 1. Seven additional QTLs (*Rbcq3*, *Rbcq4b*, *Rbcq6*, *Rbcq9a Rbcq9b*, *Rbcq11* and *Rbcq12*) were detected in an introgression line population consisting of 30 individual lines, each containing different well-defined segments of *S. habrochaites* accession LYC4 chromosomes in the genetic background of *S. lycopersicum* accession moneymaker (Finkers *et al.*, 2007b). The information generated in these studies is important for breeding programs which can identify more sources of resistance in wild relatives of other susceptible but economically important crops. If defined DNA sequences (markers) can be associated with the QTL, it is then possible to use marker-assisted selection to speed up the breeding process (Young, 1996).

The other option would be to identify genes that have a positive effect on resistance and introduce them into susceptible plants or remove those that have a negative effect (Kasuga *et al.*, 1999; Varshney *et al.*, 2005; Nurmberg *et al.*, 2007). An example of a gene with a negative effect is the MYB-related gene AS1 (ASYMMETRIC LEAVES 1) (Nurmberg *et al.*, 2007). The protein encoded by this gene is expressed in leaf founder cells, where it functions as a heterodimer with the structurally unrelated AS2 proteins to repress activity of KNOTTED 1-like homeobox (KNOX) genes. Nurmberg and associates (2007) demonstrated that loss-of-function mutations in AS1 convey increased resistance to the necrotrophic pathogens *B. cinerea* and *A. brassicicola*. Their results demonstrated that AS1 acts to suppress resistance to necrotrophs by repressing the expression of JA-induced resistance proteins. The fact that AS1 is a conserved regulator shows that this gene may be a very useful target for programs aimed at breeding for *B. cinerea* resistance.

1.10 Aim and objectives of the study

The best way to generate information about genes involved in resistance is by employing model plant species like *Arabidopsis* to study host-pathogen interactions. This information can then later be projected to cultivated crop varieties. The use of *Arabidopsis* as a model presents a lot of advantages. The genome has been completely sequenced and is publicly available (AGI, 2000; Bevan *et al.*, 2001), the plant has a relatively short life cycle of about 6 weeks from germination to maturity hence a lot of genetic information can be generated within a short time (Meinke *et al.*, 1998), it can be cultivated within a restricted area either in a greenhouse or under fluorescent lights in a laboratory (Meinke *et al.*, 1998), it is easily transformed (Alonso *et al.*, 2003) and is also host to many phytopathogens including bacteria, fungi, viruses, plant parasites and nematodes (Buell and Somerville, 1997).

Arabidopsis has been used in a number of molecular studies to generate information about host-pathogen interaction; unfortunately most of these studies have focused on biotrophic pathogens. Similarly, studies undertaken in other plants such as tomato and tobacco have focused on this group of pathogens. Biotrophic pathogens exhibit the classic *R-avr* gene interaction. In this kind of interaction, the product of an *avr* gene in the pathogen is recognized by an *R* gene product in the host leading to successful activation of defence responses and hence an incompatible interaction (Glazebrook, 2005). Downstream of this *R-Avr* protein interaction, a complex array of signalling networks has been elucidated (Martin *et al.*, 2003) with a major pathway being mediated by SA (Hammond-Kosack and Parker, 2003). Similar signalling networks are activated in response to virulent pathogens, but activation occurs more slowly after infection and it seems likely that this enables virulent pathogens to cause disease (Lamb *et al.*, 1992; Lawton *et al.*, 1996).

As explained in previous sections, the *R-Avr* system however, does not appear to impact necrotrophic pathogens and the HR, a classic *R-Avr* response, actually seems to aid infection by *B. cinerea* rather than hinder it (Govrin and Levine, 2000). A number of signalling molecules such as ET, SA and JA are synthesized in plants following recognition. SA mediates local and systemic resistance to biotrophic pathogens as well as local resistance to necrotrophs (Ferrari *et al.*, 2003). Defence against *B. cinerea* infection is primarily dependent on both JA and ET signalling

and mutations in either of these transduction pathways increases susceptibility of *Arabidopsis* and tomato (Diaz *et al.*, 2002; Ferrari *et al.*, 2003; Thomma *et al.*, 1998, 1999).

Recently, transgenic *Arabidopsis* lines overexpressing the ET response factor 1 (ERF1) a transcription factor were shown to have increased resistance against *B. cinerea* which implicates ET in this interaction (Berrocal-Lobo *et al.*, 2002). Other transcription factors that have been shown to have a role in resistance against *B. cinerea* include BOS1, WRKY33 and WRKY70. BOS1 is an R2R3MYB protein that regulates responses to both biotic and abiotic stresses (Mengiste *et al.*, 2003) while WRKY33 and WRKY7 belong to the WRKY group of transcription factors (Eulgem, 2005). WRKY proteins bind specific parts of DNA using the WRKY domain which is defined by the conserved amino acid sequence WRKYGQK (W, tryptophan; R, arginine; K, lysine; Y, tyrosine; G, glycine and Q, glutamine) at its N-terminal end. *BOS1* is up-regulated after *B. cinerea* infection and lack of this factor increased susceptibility to this pathogen. Overexpression of *WRKY33* led to enhanced resistance to *B. cinerea* and *A. brassicicola* while a loss-of-function mutation enhanced susceptibility to the two pathogens (Zheng *et al.*, 2006). WRKY70 is a node of convergence for JA- and SA-mediated signals (Li *et al.*, 2004). Overexpression of this gene resulted in constitutive expression of SA-induced genes while suppression activated JA-responsive genes (Li *et al.*, 2004). Like *WRKY33*, *WRKY70* mutants displayed increased susceptibility to *B. cinerea* (AbuQamar *et al.*, 2006). Based on this underlying information, it would be important to study gene expression profiles during *B. cinerea* infection so as to find other components of defence signalling pathways. In response to this, a gene expression profiling study was carried out. The overall aim was to enhance our understanding of the molecular events that take place during *B. cinerea* infection of *Arabidopsis* with a view of identifying candidate genes for biotechnology strategies to increase resistance. Two major studies were undertaken under this broad objective.

The main objective of the first study was to identify genes potentially involved in resistance against *B. cinerea*. A number of activities were undertaken under this objective. First, microarray experiments were carried out leading to identification of a number of genes that are differentially expressed after *B. cinerea*

infection. Using quantitative PCR, the expression profiles for a subset of genes was confirmed. Mutants developed through T-DNA insertional mutagenesis for some of these genes were obtained from the Nottingham Arabidopsis Stock Center and tested for susceptibility against *B. cinerea*. The genes expression profiles obtained in this study were also compared against expression profiles of biotrophic as well as other necrotrophic pathogens available in the public domain.

Gene expression profiling studies avail us with enormous information that is very important but does not explain biological processes in-depth. This is justified by studies that have been carried out showing a poor correlation between mRNA and protein expression, yet it is the proteins that ultimately put the information from DNA to use (Gygi *et al.*, 1999; Abbott, 1999; Ideker *et al.*, 2001; Kern *et al.*, 2003). To take this into account, a protein expression profiling study was carried out to determine the proteins that are expressed after infection of *A. thaliana* with *B. cinerea*. Two dimension - sodium dodecyl sulphate polyacrylamide gel electrophoresis (2D SDS-PAGE) and two dimension liquid chromatography (2D LC) combined with mass spectrometry were used to identify proteins expressed in this interaction.

Chapter 2

Materials and Methods

2.1 Chemicals and kits

Chemicals and kits used in the study were purchased from the following companies:

- Amersham Biosciences, Buckinghamshire, UK
- Amersham Pharmacia, Uppsala, Sweden
- Arizona University, Arizona, USA
- Bayer Garden, Bayer CropScience Limited, UK
- Beckman Coulter, Inc., Fullerton, CA, USA
- Bioline Ltd., London, UK
- Bio-Rad Laboratories Inc., Hercules, USA
- Fermentas International Inc., Ontario, Canada
- J.T. Baker, London, UK
- Invitrogen Life Technologies, Paisley, UK
- Merck Chemicals (Pty) Ltd, South Africa
- Promega Corporation, Madison, USA
- Qiagen, Crawley, UK
- Roche Molecular Biochemicals, Basel, Switzerland
- Rose Scientific, Edmonton Alberta, Canada
- Sigma-Aldrich Company Ltd., Crawley, UK

2.2 Plant material

Arabidopsis thaliana seeds of the *Columbia*-0 ecotype used in both experiments were acquired from Lehle Seeds (Lehle, Texas, USA). A comprehensive list of transgenic and mutant (T-DNA insertion lines) seed is presented in Table 2.1. Seed for T-DNA insertion lines was obtained from the Nottingham Arabidopsis Stock Centre (http://arabidopsis.info) while transgenic seed was kindly provided by various laboratories. The background for all the transgenic and mutant lines was *Columbia*-0 and the resistance phenotype, that of kanamycin resistance.

Table 2.1: List of transgenic and T-DNA insertion lines used in the study

Line	locus	Source
BGL2::GUS	At3g57260	(Bowling *et al.*, 1994)
CYP79B2::GUS	At4g39950	(Mikkelsen *et al.*, 2000)
DOGT1::GUS	At2g36800	(Poppenberger *et al.*, 2003)
OXI::GUS	At3g25250	(Rentel *et al.*, 2004)
PAL::GUS	At2g37040	(Ohl *et al.*, 1990)
N502408	At1g26400	(Alonso *et al.*, 2003)
N507462	At3g22600	"
N512997	At1g62300	"
N518193	At4g24340	"
N525603	At1g05700	"
N582089	At3g04720	"
N585809	At1g22400	"
N809256	At4g20200	(Sessions *et al.*, 2002)

2.3 Growth conditions

2.3.1 *Arabidopsis thaliana*

Seeds were stratified for four days at 4°C prior to planting. They were then planted in soil composed of peat plugs (Jiffy Products, International AS, Norway) and vermiculite mixed in a ratio of 1:1 (v/v). Planted seed was covered with cling film, to ensure high relative humidity for uniform and early germination, which was removed after one week. Fertilization of plants with plant food (Bayer Garden, Bayer CropScience Limited, United Kingdom) was done after one week and a week prior to inoculation at a rate of 1.4 g/L of water. Two weeks after planting, thinning was done and one plant left per peat plug. A temperature of 21°C, relative humidity of approximately 60% and a photoperiod of 16 hrs of light (80 - 120 μmol photons m^{-2} s^{-1}) and 8 hrs of night were maintained in the growth room. Leaf tissue used in experiments was harvested after four weeks.

2.3.2 *Botrytis cinerea*

The necrotrophic fungal pathogen *B. cinerea* (pepper isolate) which had been used in previous studies (Denby *et al.*, 2004) was used. It was maintained on commercial tinned (i.e. sterile) apricot halves (Tiger Foods, Brand Ltd, South Africa) at 25°C. Before inoculation, the apricot halves were washed in sterile water to remove fruit juice. Spores were collected 14-21 days after initial apricot inoculation.

2.4 *B. cinerea* infection

Spores were collected in 10 mL of water by gentle rubbing of the surface of developing mycelia. Pieces of mycelia were removed by filtering through glass wool. Spores were concentrated by gentle centrifugation, counted with a haemocytometer and the concentration adjusted to 500,000 spores mL^{-1} in half strength commercial grape juice (Ceres Fruit Juices (Pty) Ltd, South Africa) for leaf infection. Two methods of inoculation were tested; drop inoculations of detached leaves (detached leaf (DL) assay) and spray inoculation of whole plants (whole plant (WP) assays). For DL assays, *Arabidopsis* leaves were placed on trays containing 0.8% (w/v) agar. Depending on the size of the leaf, 8-10 drops of 10 μL spore suspension were placed on top of each leaf using the Eppendorf® Multipette Plus (Eppendorf AG, Hamburg, Germany). Control leaves were treated with half strength grape juice containing no spores. For WP assays, whole plants were sprayed with a spore suspension of the same concentration until the droplets ran off. Control plants were sprayed with half strength grape juice containing no spores. DL assays were used for both microarray and proteomics studies. All leaf tissue for these two studies was infected at the same time of the day, on non-bolting plants of the same age (4 weeks). Infected and mock treated leaves were harvested, frozen in liquid nitrogen and kept at -70°C until needed.

DL and WP assays were used in another experiment involving T-DNA insertion lines. For DL assays, three leaves from each mutant plant were inoculated with a drop of 10 μL of spore suspension placed in the middle of each leaf. Images of the inoculated T-DNA leaves were acquired on scale using a Fujifilm digital camera (Fujifilm, USA) at 2 and 3 days post inoculation (dpi). A 1 cm scale was included

in each image to enable calibration of the measurements. The acquired images were imported into ImageJ software (Abramoff *et al.*, 2004) and using this software, the diameter of the lesion for each mutant leaf was measured. ImageJ is freely available at http://rsb.info.nih.gov/ij. Data was statistically analyzed using analysis of variance (ANOVA) with Genstat (Genstat, VSN International, Hemel Hempstead). For WP assays, whole mutant plants were sprayed as already described; symptoms were scored on the 2^{nd}, 3^{rd} and 4^{th} day after inoculation using the following scale: no/very few necrotic spots, 1; significant chlorosis, 2; necrotic spots on most leaves, 3; fungal hyphae visible by eye, 4; and extreme fungal growth, 5. The wild-type used in this experiment was from the same background as the mutants. It was obtained from the analyzed mutant set and confirmed with polymerase chain reaction (PCR) (Section 2.14). In all cases, trays containing inoculated leaves and plants were covered with cling film or a similar sized tray. This was to maintain high relative humidity so as to reduce the rate of evaporation of spore-containing droplets.

2.5 Camalexin measurement

Leaves were harvested, frozen in liquid nitrogen and kept at -70°C until use. Whole leaf tissue equivalent to 80 mg was transferred to 500 μL of 80% (v/v) methanol and heated at 65°C for 20 min. The methanol extract was transferred into a clean tube and evaporated under vacuum to approximately 100 μL. An equal amount of chloroform was added to the methanol solution and mixed well with a vortex mixer. The chloroform extract was transferred into a clean tube and the process repeated. The residue was dissolved in 10 μL of chloroform, applied to a silica TLC plate (J.T. Baker/VWR Scientific) and developed in 9:1 (v/v) chloroform/methanol for 45 min. Camalexin was visualized under a long wavelength UV filter using Gene Genius Bio-Imaging System (Syngene Inc, Cambridge, UK).

2.6 Histochemical staining for H_2O_2 accumulation

Leaves were incubated in 2 mL 1 mg/mL 3,3-diaminobenzidine (DAB) (Sigma, Crawley, UK) and gently shaken at room temperature for 2-4 hrs until a reddish-brown precipitate could be seen. Chlorophyll in stained leaves was then removed with

several washes of 70% (v/v) ethanol. Images of stained leaves were obtained by scanning the leaves with a Canonscan 8400F Scanner (Canon, Lake Success, New York, USA).

2.7 Histochemical staining for GUS activity

Leaves were immersed in β-glucuronidase (GUS) staining solution (10 mM EDTA, 100mM Na_3PO_4 pH 7.0, 0.5 mM $K_4Fe(CN)_6$, 0.1% (v/v) Triton X-100 and 50 mg/mL X-Gluc) and incubated overnight in the dark at 37°C. To stop the reaction, leaves were washed several times with 0.1 M Na_3PO_4 pH 7.0, followed by several washes with 70% (v/v) ethanol to remove chlorophyll. Images were obtained by scanning the leaves with a Canonscan 8400F Scanner (Canon, Lake Success, New York, USA).

2.8 Gene expression profiling using microarrays

2.8.1 Experimental design

Two microarray experiments were carried out. In the first experiment (time course), four week old *Arabidopsis* leaves were inoculated with *B. cinerea* and harvested after 12 and 24 hpi. Mock inoculated leaves were treated with half-strength grape juice but with no *B. cinerea* spores. Five biological replicates (independently grown and inoculated plants) were conducted in this experiment. Biological comparisons were made between mock treated and inoculated leaves. In the first replicate, cDNA from mock treated and infected leaves was labeled with the red dye, cyanine 5 (Cy5) and the green dye, cyanine 3 (Cy3) respectively while in the last four replicates (rep 2-5), cDNA from mock treated leaves was labeled with Cy3 while that from infected leaves was labeled with Cy5 (Table: 2.2). Therefore the first replicate was essentially a dye swap. Dye swaps are very important in reducing systematic dye bias (Tseng *et al.*, 2001; Yang *et al.*, 2001).

In the second experiment (spatial), four-week *Arabidopsis* leaves were inoculated with a single drop of *B. cinerea* spore suspension in the middle of the leaf. A similar treatment was made for control leaves but with half-strength grape juice without *B. cinerea* spores. Using a cock borer of 6 mm in diameter, leaf disks were

cut from the edge of the lesion (0-6 mm) and exactly after the first cut (6-12 mm) after 48 hpi. This experiments was conducted in three biological replicates. Biological comparisons were made between mock treated leaf disks and disks (0-6 and 6-12 mm) from inoculated leaves. In the first two replicates, cDNA from disks cut from mock treated leaves was labeled with cy3 while cDNA from disks cut from treated leaves was labeled with cy5. In the last replicate, the dyes were swaped (Table: 2.2). In all experiments a reference design in which treatments are compared to a common reference was used (Quackenbush, 2001; Yang and Speed, 2002).

2.8.2 RNA extraction

One gram of tissue was ground in liquid nitrogen and transferred to the extraction buffer (100 mM Tris-HCl pH 9.0, 200 mM NaCl, 5 mM dithiothreitol (DTT), 1% (v/v) Sarcosyl and 20 mM EDTA in DEPC treated water). Equal volumes of phenol (3 mL) and chloroform: isoamyl alcohol (CI) (3 mL) were added and the solution was mixed by vortexing. It was centrifuged at 9,447 x g for 10 min. The aqueous phase was recovered and extracted with an equal volume of CI. After centrifugation at 9,447 x g for 10 min, one third volume of 8 M LiCl solution was added to the aqueous phase and allowed to precipitate overnight at 4^{o}C. The next day, RNA was pelleted by centrifuging at 9,447 x g for 10 min followed by washing in 500 μL of 2 M LiCl. The RNA pellet was dissolved in DEPC treated water and precipitated with 1/10 volume 3 M sodium acetate, pH 5.2 and 2.5 volumes of absolute ethanol at -20^{o}C overnight. The following day, RNA was re-pelleted, washed with 70% (v/v) ethanol and resuspended in DEPC treated water. Total RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware) and separated on a 1.0% (w/v) agarose-formaldehyde gel to assess integrity.

2.8.3 Synthesis of cDNA

Reverse transcription reaction was performed using 25 μg total RNA. 17.5 μL of the RNA sample and 2 μL of OligodT (50 μg/mL) were mixed, heated for 10 min at 70^{o}C and chilled on ice for 10 min. After centrifugation for 30 sec at 9,477 x g, 10.5 μL of the reverse transcription master mix (25 mM each of dATP, dCTP and

dGTP; 15 mM dTTP; 10 mM aminoallyl-dUTP: 0.1 M DTT; 5X RT reaction buffer and SuperScript III reverse transcriptase (200U/μL) (Invitrogen, Carlsbad)) were added to a total volume of 30 μL. Reverse transcription was carried out at 46^{o}C overnight. Following cDNA synthesis, RNA was degraded by adding 10 μL each of 1 M NaOH and 0.5 M EDTA (pH 8.0) to the mix and incubated at 65^{o}C for 15 min. The reaction was neutralized with 10 μL of 1 M HCl. Newly synthesized cDNA was purified using RNeasy mini columns (Qiagen, Valencia, CA) based on manufacturers instructions. The concentration of cDNA was determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware).

2.8.4 Preparation of labeled probes

Samples of cDNA were dried under vacuum, resuspended in 5 μL of 0.1 M $NaHCO_3$, coupling buffer followed by addition of 5 μL of Cy3 and Cy5 (Amersham Pharmacia, UK). Cy3 and Cy5 which are provided as a dried product were resuspended in dimethyl sulfoxide (DMSO), aliquoted and stored at -20^{o}C. In the coupling reaction, the terminal amino group on the aminoallyl-dUTP reacts with the N-hydroxysuccinimide(NHS)-monoester dye to form a Cy-aa-UTP covalent bond. This reaction is performed at pH 8.5-9.0, which is an optimal balance between deprotonation of the amino group and nucleophilic attack and hydrolysis reactions of the NHS-monoester ester with water. $NaHCO_3$, also referred to as the carbonate-bicarbonate buffer, is preferred because it is more stable and easier to prepare than other buffers. However, its composition changes overtime due to its decomposition into carbon dioxide and water. Thus $NaHCO_3$ prepared within the last four weeks was used. The labeling reaction was incubated for 1 hr in the dark because Cy dyes are light sensitive; mixing every 15 min. Unincorporated dyes from Cy3 and Cy5 reactions were removed using RNeasy mini columns (Qiagen, Valencia, CA). The column eluates were combined and the fluorescent labelling verified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware). Light absorption at 260, 550, and 650 nm was measured to determine the concentration of cDNA and incorporated Cy3 and Cy5 respectively.

Table 2.2: Hybridizations performed in the two microarray experiments

Rep[a]	Number	Slide label[b]	Cyanine 3	Cyanine 5	Experiment
1	1	AT.v3.01.02.075	24 hrs treated	24 hrs mock	Time course
1	2	AT.v3.01.02.076	12 hrs treated	12 hrs mock	"
2	3	AT.v3.01.02.083	12 hrs mock	12 hrs treated	"
2	4	AT.v3.01.02.084	24 hrs mock	24 hrs treated	"
3	5	AT.v3.01.04.255	12 hrs mock	12 hrs treated	"
3	6	AT.v3.01.04.266	24 hrs mock	24 hrs treated	"
4	7	AT.v3.2.2.184	12 hrs mock	12 hrs treated	"
4	8	AT.v3.2.2.189	24 hrs mock	24 hrs treated	"
5	9	AT.v3.2.2.177	12 hrs mock	12 hrs treated	"
5	10	AT.v3.2.2.181	24 hrs mock	24 hrs treated	"
1	1	AT.v3.2.2.178	0-6 mm mock	0-6 mm treated	Spatial
1	2	AT.v3.2.2.179	6-12 mm mock	6-12 mm treated	"
2	3	AT.v3.2.2.180	0-6 mm mock	0-6 mm treated	"
2	4	AT.v3.2.2.182	6-12 mm mock	6-12 mm treated	"
3	5	AT.v3.2.2.186	0-6 mm treated	0-6 mm mock	"
3	6	AT.v3.2.2.183	6-12 mm treated	6-12 mm mock	"

[a]Replicate

[b]In the slide label, AT v3 XX.YY.ZZZ; XX refers to the lot number of the oligonucleotides used for printing. YY refers to the print run number, and ZZZ refers to the slide number within the print run (from 1-308). (Source: www.ag.arizona.edu/microarray).

2.8.5 Hybridization, wash and scan

The Galbraith laboratory operon long oligonucleotide microarray slides (version 3) for *Arabidopsis* were used in this study (www.ag.arizona.edu/microarray). They were first rehydrated over a 60°C water bath for 10 sec by holding the labeled side over water vapour; snap dried on a 65°C heating block for 5 sec and allowed to cool for 1 min. This process was repeated 5 times. The slides were then UV cross-linked by exposing them (label-side-up) to 180mJ UV. Cross-linked slides were washed in 1% (v/v) SDS for 5 min. SDS was removed by dipping the slides 10 times in milli-Q water and 5 times in absolute ethanol. They were spin dried in a centrifuge at 9,447 x g for 4 min. The two purified labeled probes were concentrated by centrifugation under vacuum, quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware) and mixed in equal concentrations of labeled cDNA. The mixed probes were centrifuged at 9,447 x g for 1 min and the hybridization solution consisting of 20 μL of 20X SSC, 12 μL of 10% (v/v) BSA,

8 μL of SDS added. Milli-Q water was also added to make to a total volume of 200 μL. The labeled probe was denatured by placing in boiling water for 2 min and transferred immediately to ice. The microarray slide was placed on a 65^{o}C heating block (label-side-up); the labeled probe was applied and covered with a coverslip avoiding trapping any air bubbles. The slide was incubated in a sealed chamber in a 55^{o}C water bath for 8-12 hrs. After hybridization, the slides were washed in 2X SSC, 0.5% (v/v) SDS for 5 min at 55^{o}C followed by 0.5X SSC for 5 min and 0.05X SSC for 5 min. The last two washes were carried out at room temperature. The slides were then scanned for fluorescence emission using a GenePix® scanner 4200A (Axon Instruments, Union City, CA).

2.8.6 Data analysis

Images were analyzed with Genepix version 5.1 (Axon Instruments, Union City, CA) to generate fluorescent and background intensity values for each spot in both the Cy3 (green) and Cy5 (red) channels. Spots that were poorly segmented by the GenePix Pro 5 software were manually corrected. GenePix results files (GPR) were uploaded into the gene expression pattern analysis suite (GEPAS) software (Herrero *et al.*, 2003) for normalization. GEPAS is a web-based resource for microarray gene expression data analysis and is freely available at http://gepas.bioinfo.cipf.es. To correct for spatial and intensity dependent patterns within and between microarray slides, print-tip locally weighted scatterplot smoothing (LOESS) normalization was used. Replications within slides were merged based on average log transformed ratios. Oligoslides 1 and 2 (time course experiment) and 5 and 6 (spatial experiment) were multiplied by -1 because they were dye swaps (Table 2.2). All genes having more than two missing values across the experiment were not considered for significance analysis.

Using the normalized log ratios for each array, differentially expressed genes were determined using a one class t-test of the significance analysis of microarray package (Tusher *et al.*, 2001) which is available in TMEV. TMEV is a component of the TM4 microarray software suite (Saeed *et al.*, 2003) which is freely available at http://www.tm4.org. Significance analysis of microarray requires two user set parameters; a minimal fold change value and a threshold value that can be adjusted

to maximize the number of significant genes while minimizing the predicted false discovery rate (FDR). A 5% FDR was considered for the analysis. Once the data set was generated, genes were selected based on fold change (cut-off of $\log_2$ of 1 i.e. 2 fold) and statistical significance. Gene expression changes at 24 hpi were also depicted in MapMan format version 2.1.1 (Thimm *et al.*, 2004).

2.9 Quantitative PCR

Total RNA equivalent to 5 μg was denatured at 70°C for 10 min followed by a quick chill on ice in a 12 μL reaction volume containing 1 μL of Oligo (dT) (500 μg/mL) and 1 μL of 10 mM dNTPs. After the addition of 4 μL of 5x reaction buffer (Invitrogen, Carlsbad) and 2 μL of 0.1 M DTT, the reaction was preheated to 46°C for 2 min before adding 1 μL (200 units) of Superscript III reverse transcriptase (Invitrogen, Carlsbad) followed by an overnight incubation. The reaction was terminated by heating at 70°C for 15 min. The final reaction mix was diluted in water to a volume of 200 μL. 10 μL of each cDNA sample were pooled together, quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware) and then serially diluted (1:10, 1:100, 1:1000) for standard curve generation. Primers (Table 2.3) were designed using Primer3 (Rozen and Skaletsky, 2000) which is freely available at http://frodo.wi.mit.edu/ and checked in DNAMAN (Lynnon Corporation) for complementarity. All amplification conditions were optimized on a normal PCR machine before proceeding with quantitative PCR.

Quantitative PCR reactions were carried out in a total volume of 25 μL containing 5 μM of each primer (forward and reverse), 1X SYBR green PCR master mix (Quantace, London) and 1 μL of cDNA template. Amplifications were carried out with the Rotor-Gene^{TM} 3000 Real-time Multiplexing System (Corbett Research, Sydney). The PCR program consisted of an initial denaturation step at 95°C for 10 min followed by 40 cycles of 95°C for 15 sec, 55°C for 10 sec and 72°C for 20 sec. A melting curve was produced to confirm a single gene-specific peak and to detect primer-dimer formation by heating the sample from 72°C to 95°C in 1°C increments with a waiting time at each temperature of 5 sec. Four housekeeping genes; At5g44200 (Nuclear cap-binding protein), At5g25760 (Ubiquitin ligase), At5g06600

(Ubiquitin-specific protease) and At1g04820 (Tubulin) were selected based on microarray results. These internal controls were identified as those that did not vary significantly during both time points. All real-time PCR reactions were conducted in triplicates. Quantification was based on cycle threshold (Ct) values.

2.10 Functional categorization

To determine over-represented gene ontology functional categories in genes up-regulated in the time course experiment after 24 hpi, two lists of genes were compiled. The first list contained all genes significantly up-regulated while the second list contained all *Arabidopsis* genes but without the up-regulated genes. The two lists were uploaded into FatiGO, (http://fatigo.bioinfo.cipf.es) (Ashburner *et al.*, 2000).

2.11 Promoter motif analysis

Genes significantly differentially up-regulated after 12 and 24 hpi were divided into three categories; those specific at 12 and 24 hpi and those overlapping in the two time points. Each list was uploaded into motif finder (http://www.arabidopsis.org/tools/bulk/motiffinder) to such for over-represented 6-mer elements in the 500 bp sequences upstream of the ATG start codon. The over-represented elements were compared with known plant motifs recorded in PlantCare (http://bioinformatics.psb.ugent.be/webtools/plantcare) (Rombauts *et al.*, 1999) and PLACE (http://www.dna.affrc.go.jp/PLACE) (Higo *et al.*, 1999) databases.

2.12 Cluster analysis

Microarray data from other experiments were downloaded from public repositories (gene expression omnibus and AtGenExpress). All arrays were normalized, organized in a log with columns representing experiments and rows representing genes and subsequently clustered using hierarchical clustering and SOTA in TMEV. The genevestigator database (Zimmermann *et al.*, 2004) was also utilized to compare expression profiles of genes induced by *B. cinerea* after 24 hpi in other treatments.

Table 2.3: List of primers used for quantitative PCR. The size of the expected PCR fragment for each gene is shown in the last column

Gene locus	Description	Forward primer	Tm (°C)	Reverse primer	Tm (°C)	size(bp)
At1g04820	Tubulin alpha-2/alpha-4 chain	5′-CGTGCTTTCGTTCACTGGTA-3′	60.4	5′-CAAGATCCTCACGTGCCTCT-3′	59.9	73
At1g12900	Glyceraldehyde 3- phosphate dehydrogenase	5′-AACTGTCTCGCTCCATTCGT-3′	59.9	5′-CTGCTGCTCTTGCTCTCCTT-3′	60.0	142
At1g20340	Plastocyanin	5′-GGCCTTAAAGCCTCAACCAT-3′	60.5	5′-CGCGACTCCGAAGTTCTTTA-3′	60.5	111
At1g22400	UDP-gluconosyl transferase family protein	5′-ATGGTGTGTTGGCCATTTT-3′	60.1	5′-ACCGCCTCAACTTCCTCTCT-3′	60.4	116
At1g52200	Expressed protein	5′-CTGGGTGATGGGATCAAAGT-3′	59.8	5′-GCAACAAGGGCATAGGACAT-3′	60.0	103
At1g62300	WRKY family transcription factor	5′-CTACCCCGACGACATTTGAT-3′	59.8	5′-ATTCCCTTTGGCCATCTTCT-3′	60.0	140
At1g72610	Germin-like protein	5′-CATTACCGCTGGGTTTGTCT-3′	60.0	5′-CATGACCTGTCCTGGTTTGA-3′	59.5	70
At2g24180	Cytochrome P450 family protein	5′-GATTGGAAAGCGACTGAGGA-3′	60.3	5′-GAGGATGCTTCCTGTGGCTA-3′	60.4	70
At2g39030	GCN5-related N-acetyltransferase	5′-GTGGCTGGATTCGTTCTGTT-3′	60.1	5′-GTTTTCCGAAGCCTTTTCGT-3′	60.6	110
At2g47190	Myb family transcription factor	5′-GGATGCCGAGATTAGTGGAA-3′	60.0	5′-GACTTGGGTCGGTGATCATT-3′	59.8	87
At3g04220	Disease resistance protein	5′-TCCGCAAAGACTTCCTCAGT-3′	60.0	5′-TAATCGCCCGAATGAGTTCT-3′	59.7	120
At3g04720	Hevein-like protein	5′-TCCGACCAACAACTGTCAGA-3′	60.3	5′-CGCCGGATTATAGAAATGGT-3′	58.9	102
At3g28930	avrRpt2-induced AIG2 protein	5′-GTCATGGTTTCCGCTCAACT-3′	60.1	5′-TCCATTGTCGGAAGGAGAAA-3′	60.6	84
At3g50480	Broad-spectrum mildew resistance	5′-GGAGCCCAAGTCATTTACGA-3′	60.1	5′-GTACGGCCGAGACGGTTAT-3′	61.3	71
At3g51660	Macrophage migratory inhibitory protein	5′-GTCGGACGACCTCAAAACTT-3′	59.2	5′-GCCTCCCATTGACACAATCT-3′	60.0	111
At4g10340	Chlorophyll-A-B binding protein	5′-GCCGTAGTTGCTGAGGTTGT-3′	60.3	5′-GGGTGTAGCTTGTCCTCGAA-3′	60.2	86
At4g15610	Integral membrane family protein	5′-CCTTGGTCTGAAGGGAAACA-3′	60.1	5′-GGACAACCGAAGCAAACAAT-3′	60.0	116
At4g16260	Glycosyl hydrolase family 17 protein	5′-CCATCCTCAACCCAACAAGT-3′	59.8	5′-GGCTTGGTTTGGATCGTAGA-3′	60.1	143
At4g24340	Phosphorylase family protein	5′-CCATGACTTGCGTGTCAAAT-3′	59.6	5′-CAGTGTTAGTGGCAGCCAAA-3′	60.0	117
At4g30270	MERI-5 protein	5′-CAATCCTCTGGAACCCTCAA-3	60.0	5′-CATCCTCATCGGCTTGTTCT-3′	60.2	117
At4g33150	Lysine-ketoglutarate reductase	5′-TTGCATTGGAGTGTTTTCCA-3′	60.1	5′-GTTGTTGCTTCGCTCTCGAT-3′	60.6	76
At5g06600	Ubiquitin-specific protease 12	5′-GCAAAACACCAGGAAGCATT-3′	60.1	5′-CAAACTCGCAGCATCAGAAA-3′	60.1	133
At5g06860	Polygalacturonase inhibiting Protein 1	5′-CCAACTGGAGGGAAACTTCA-3′	60.1	5′-CAAGAGGAGCACCACACAAA-3′	59.9	134
At5g07010	Sulfotransferase family protein	5′-CCACCCTCTTTTCACTTCCA-3′	60.1	5′-GACTGGCTAGACCCGAGAGA-3′	59.6	101
At5g25760	Ubiquitin-conjugating enzyme	5′-GGACCGCTCTTATCAAAGGA-3′	59.2	5′-CTTGAGGAGGTTGCAAAGGA-3′	60.4	102
At5g38430	Ribulose biphosphate carboxylate	5′-CGTCGAATTGGCTAAGGAAG-3′	59.8	5′-CGTGCTCACGGTACACAAAT-3′	59.6	98
At5g39610	No Apical Meristem family protein	5′-CAGCCGGTTTACCTTCGTTA-3′	60.1	5′-GTTTCTTGGTCGGAGAAGCA-3′	60.4	91
At5g44200	Nuclear cap-binding protein (CBP)	5′-CCTTGTGGCTTTTGTTTCGT-3′	60.2	5′-GCCCCATTGTCTTCCTTCTT-3′	60.4	141

2.13 Extraction of genomic DNA

A four-week old leaf was homogenized in 500 μL of superquick extraction buffer (200 mM Tris-HCl pH 7.5, 250 mM NaCl, 25 mM EDTA pH 8.0 and 0.5% SDS) (Edwards *et al.*, 1991) followed by incubation at 60°C for 10 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, vortexed and centrifuged at 9,447 x *g* for 10 min. The supernatant was transferred into a new tube. DNA was precipitated with 0.1 volume 3 M sodium acetate pH 5.2 and 0.7 volume isopropanol and pelleted by centrifuging at 9,447 x *g* for 10 min. The supernatant was removed and the pellet washed with 70% (v/v) ethanol by vortexing and centrifuged at 9,447 x *g* for 5 min. The pellet was resuspended in 100 μL of 1X TE buffer (10 mM Tris-HCl pH 7.5 and 1 mM EDTA pH 8.0).

2.14 PCR selection of T-DNA insertion lines

Two paired PCR reactions were set up to select homozygous mutant lines using primers designed at http://signal.salk.edu/tdnaprimers. One set consisted of gene specific primers (RP+LP) while the other set consisted of a gene specific right primer and the T-DNA insertion left border primer (RP+BP). All primers used in this selection are presented in Table 2.4. A product from the first set with no product in the second set indicated wild-type genotype, while a product from the second set with no product from the first set indicated homozygous mutant however, a product from both sets indicated heterozygosity. PCR reactions were carried out in a total volume of 50 μL, containing 5 μL of 10X PCR buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 1.5 μL of 50 mM $MgCl_2$, 1 μL of 10 mM dNTP mix, 1 μL of 5 μM each primer, 3 μL of genomic DNA and 2 μL *Taq* DNA polymerase (5U/μL). Reactions were amplified in a GeneAmp PCR System 2700 thermocycler (Applied Biosystems, Foster City, CA, USA). The thermal cycling programme consisted of a pre-denaturation step at 95°C for 5 min and 35 cycles of 94°C for 1 min, 54°C for 1 min and 72°C for 1 min followed by a final extension at 72°C for 10 min. The amplified products were subjected to electrophoresis on 1.0% agarose gel in 1X TAE buffer, stained with ethidium bromide and visualized under UV light.

Table 2.4: Primers used in the selection of homozygous T-DNA insertion lines.

T-DNA line	Gene locus	Primer	Tm (°C)	LP+RP (bp)	BP+RP (bp)
N502408	At1g26400	5′-TGCGGAGTTTAGGAAACCAAG-3′	61.5	1023	434-734
		5′-CATGGTAGCTCGATCCAAGAG-3′	59.8		
N505635	At1g15520	5′-TTTATCGAATGCAATGGGTTC-3′	59.8	977	431-731
		5′-AATCCAGTGTTGTTGATCATCC-3′	58.8		
N507462	At3g22600	5′-CTCACGGTTGTTCTGTTTTGG-3′	60.3	1045	502-802
		5′-ATGTAGTTGAGACACGGCGAC-3′	60.2		
N512997	At1g62300	5′-ATCTTACTTGTTTGCGAACGG-3′	59.3	1035	462-762
		5′-TTTGATAAAGGTATCATATTTTGTTTTC-3′	57.5		
N518193	At4g24340	5′-TATGCATTAAACATGAGGGGC-3′	59.8	973	445-745
		5′-CAGTGGTCACATCAATTCACC-3′	58.8		
N525603	At1g05700	5′-ATATATGACCCGTTAACCCGC-3′	60.0	971	445-745
		5′-TACGCTCTCTCATGGGTTCAC-3′	60.3		
N582089	At3g04720	5′-AACAATGAGATGGCCTTGTTG-3′	60.0	977	436-736
		5′-TCTATCGAATCCAACGAAATTTC-3′	59.5		
N585809	At1g22400	5′-ACCTCCGGTTTATTCAGTTGG-3′	60.2	973	435-735
		5′-GATAATGATAGCATTGCATGCC-3′	59.5		
N809256	At4g20200	5′-TTTCACTTGACGTCATTTCCC-3′	60.0	980	444-744
		5′-TAACTTGAAGCTGAGCTTGGC3-′	59.8		

LP, RP and BP stand for gene specific left and right primers and T-DNA border primer respectively. LP+RP represents the size of the PCR fragments expected when gene specific primers are used while BP+RP represents the size of the PCR fragment expected from a line carrying the insertion.

2.15 Protein expression profiling

2.15.1 Proteome analysis using 2D SDS-PAGE

2.15.1.1 Protein sample preparation

Leaf tissue weighing 250 mg was ground in liquid Nitrogen and transferred to 500 μL ice-cold protein extraction buffer (0.5 M Tris-HCl pH 7.5, 10 mM EDTA, 1% (v/v) Triton X-100, 2% (v/v) 2-mercaptoethanol, 1 mM PMSF). The mixture was votexed and centrifuged at 9,447 x g for 5 min at 4^{o}C. The supernatant was transferred to a new 2 mL tube and the protein concentration determined using the Bradford assay (Bradford, 1976) with a DU® 650 spectrophotometer (Beckman). Aliquots of the total extract equivalent to 250 μg of protein from various treatment samples were used in subsequent steps. An equal volume of ice cold phenol pH 8 was added to the supernatant and the mixture vortexed for 10 sec followed by centrifugation at 9,447 x g for 1 min. This allowed the phases to separate and the proteins were seen as a white milky interface which was not disturbed. Approximately 80% of the upper aqueous phase were discarded and the volume replaced by addition of extraction buffer. The mixture was vortexed for 10 sec and centrifuged at 9,447 x g for 1 min at 4^{o}C. The upper aqueous phase was again removed and discarded. The proteins were precipitated by adding 5X volume of 0.1 M ammonium acetate in methanol and incubated overnight at -20^{o}C. The following day the samples were centrifuged at 9,477 x g for 5 min at 4^{o}C and the supernatant discarded. The pellet was washed in 0.1 M ammonium acetate in methanol followed by a wash with 80% acetone. The pellet was then air dried for 30 min in the fume hood.

2.15.1.2 Rehydration of the IPG strip

The final pellet was resuspended in 155 μl of sample rehydration buffer (8 M Urea, 2 M thiourea, 2% (w/v) CHAPS, 2% (v/v) IPG buffer , 2% (v/v) Triton X-100, 0.3% (w/v) DTT and trace bromophenol blue) by pipetting and vortexing. The samples were incubated for 1 hr at room temperature with agitation. Heating the sample under any circumstances was avoided to prevent carbamylation of proteins. The ZOOM® IPGRunner™ cassette (Invitrogen Life Technologies, Paisley, UK) was

then set on a level surface with the sample loading wells facing upwards. The whole protein sample contained in the rehydration buffer was loaded into each sample loading well. A 7 cm IPG narrow-range strip pH 4-7 (Bio-Rad, Laboratories Inc, Hercules, USA) was held at the basic (-) end using a forceps with the gel side up and guided gently into the enclosed channel through the sample loading well at the curved end of the cassette avoiding development of air bubbles. The strip was slid until the acidic (+) end touched the end of the channel slot. Any air bubbles that developed were removed by sliding the strip gently back past the bubble and then back in. The process was repeated until all the strips had been loaded. The sample loading wells were sealed with tape and incubated overnight at room temperature on a flat surface.

2.15.1.3 Isoelectric focusing

The cassette was then assembled into the ZOOM® IPGRunner mini-cell (Invitrogen Life Technologies, Paisley, UK) as per manufacturer's instructions. Deionized water was used as the running buffer. Sealing tape and two sample loading devices were removed and replaced with an electrode wick at each end of the ZOOM® IPGRunner™ cassette. Approximately 600 μL of deionized water was applied to each end of the electrode wicks. Isoelectric focusing was performed using a step voltage protocol of 200 V for 20 min, 450 V for 15 min, 750 V for 15 min and 2,000 V for 105 min. Following isoelectric focusing, the strips were immediately placed into 15 mL of equilibration buffer I (6 M urea, 0.375 M Tris pH 8.8, 2% (w/v) SDS, 20% (w/v) glycerol and 2% (w/v) DTT) and gently shaken for 10 min at room temperature. The strips were subsequently transferred to 15 mL of equilibration buffer II (6 M urea, 0.375 M Tris-HCl pH 8.8, 2% (w/v) SDS, 20% (w/v) glycerol and 2.5% (w/v) iodoacetamide and trace bromophenol blue) and gently shaken for 10 min at room temperature. The first equilibration reduces the proteins while the second alkylates them. Reduction and alkylation steps are included in the protocol to prevent the formation of mixed disulphides and disulfide bridge reformation respectively.

2.15.1.4 2D SDS-PAGE

A 12% (v/v) polyacrylamide gel consisting of a separating and stacking gel was set up in a 20 x 10 cm gel system (Bio-Rad Laboratories, Inc. Hercules, USA). The separating gel consisted of 12% (v/v) acrylamide, 375 mM Tris-HCl pH 8.8, 0.1% (w/v) SDS, 0.1% (w/v) ammonium persulphate and 0.05% (v/v) TEMED and was over-layed with a stacking gel of 5% (v/v) acrylamide, 125 mM Tris pH 8.8, 0.1% (w/v) SDS, 0.09% (w/v) ammonium persulfate and 0.1% (v/v) TEMED. The equilibrated IPG strips were loaded on top of the stacking gel and sealed with 0.5% (w/v) agarose made in 1X running buffer (25 mM Tris pH 8.8, 192 mM glycine and 0.1% (w/v) SDS) and allowed to set. The second dimension was electrophoresed in a Mini-PROTEAN® 3 Dodeca Cell (BioRad, Laboratories Inc, Hercules, USA) which was set at an initial voltage of 100 V for 30 min, followed by 150 V for 1-2 hrs until the bromophenol blue dye front had completely migrated out of the gel. Each mini gel was immediately fixed by incubating in 100 mL fix solution (50% (v/v) methanol and 10% (v/v) acetic acid) with gentle shaking for 30 min followed by an overnight incubation in fresh fix solution. The next day, the gels were washed three times with 100 mL sterile distilled water and stained immediately.

2.15.1.5 Staining for proteins

To determine the total amount of proteins, gels were stained with colloidal coomassie (1% (v/v) coomassie G250 stock, 3% (v/v) orthophosphoric acid and 6% (v/v) ammonium sulphate) for 48 hrs at room temperature. They were then destained with 25% (v/v) methanol for less than 5 min and rinsed with sterile distilled water.

2.15.1.6 Image acquisition and data analysis

Digital images of the stained gels were captured using UVIpro gel documentation system (UVItec Limited, Cambridge, UK). The acquired images in the TIFF format were imported into PDQuest® software, version 7.2.0 (Bio-Rad, Laboratories Inc, Hercules, USA). Default settings were used during automated spot detection and matching prior to manual inspection and any necessary editing of spot matching. Alignment of spots in all images in a match set was based on a number of selected

landmarks. The built in method of total density in gel image was used for normalization. After pooling replicates, the relative change in protein levels between the treatment and the control was represented by the ratio between them. Spots showing $\geq$ 2-fold induction or suppression compared to control treatment were considered to be differentially regulated. Most of these selections were also statistically significant (n=5, $P < 0.05$) after the students t-test.

2.15.2 Proteome analysis using 2D liquid chromatography

2.15.2.1 Protein sample preparation

One gram of mock treated and infected *Arabidopsis* leaf tissue was ground in liquid nitrogen to a fine powder. For each 0.5 mL of fine powder, 2 mL of lysis denaturing buffer (5 M urea, 2 M thiourea, 10% (v/v) glycerol, 50 mM Tris-HCl (pH 7.8-8.2, between 10-25^{o}C), 2% (w/v) n-octylglucoside (octyl α-D-glucopyranoside), 2.5% (w/v) SB3-10, 5 mM TCEP and 1 mM protease inhibitor cocktail) was added. All components used in the preparation of this buffer were purchased from Sigma-Aldrich Gillingham, Dorset, UK. The mixture was vortexed for 1 min, sonicated for 5 min and finally centrifuged at 13,000 x g for 60 min. The whole extraction procedure was carried out at room temperature to avoid precipitation of the lysis buffer. The supernatant was transferred to a new 25 mL tube and stored at -20^{o}C until needed for processing. The PD-10 desalting column (Amersham Biosciences, Buckinghamshire, England) was equilibrated with 25 mL of protein fractionation start buffer (Beckman Coulter, Inc., Fullerton, CA). A volume of 2.5 mL of the supernatant for each sample was loaded on to the equilibrated PD-10 desalting column and the eluent discarded. The column was then washed with start buffer to elute the proteins and the first 3.5 mL fraction was collected. The protein concentration of the eluent was determined by the Bradford assay (Bradford, 1976) with an 8452A diode array spectrophotometer (Hewlett Packard). Five milligrams of total protein from each sample was removed and diluted in the start buffer to bring the volume for each sample to 7 mL.

2.15.2.2 Chromatofocusing

Proteins were separated in the first dimension by chromatofocusing performed on a HPCF-1D column (250 x 2.1 mm) (Beckman Coulter, Inc., Fullerton, CA). In chromatofocusing proteins are bound to a strong anion exchanger followed by elution with a continuously decreasing pH gradient (8.5-4.0) so that proteins elute in order of their isoelectric points. This pH gradient was generated on the column by two buffers; the start and eluent buffers (Beckman Coulter, Inc., Fullerton, CA). Before use, the start and eluent buffers were calibrated by sonication for 5 min followed by pH adjustment to 8.5 and 4.0 respectively using either a saturated solution of 50 mg/mL iminodiacetic acid if the buffer was too basic or 1 M ammonium hydroxide if it was too acidic. The chromatofocusing column was then equilibrated with calibrated start buffer to an initial pH 8.5 for 210 min at a flow rate of 0.2 mL/min.

Five milligrams of total protein from each sample were diluted in start buffer to a total volume of 7 mL. The sample was then manually injected onto the chromatofocusing column for first dimension separation. The start buffer was pumped through the column for the first 35 min to elute proteins with pI values above 8.5. After 35 min, a pH gradient from 8.5 to 4.0 was started by introduction of the eluent buffer (pH 4.0) and this continued up to 130 min. After the pH gradient, the most acidic proteins were recovered by washing the column with 1 M sodium chloride for 25 min. This was followed by a final wash in water for 45 min. The pH of the effluent from the first dimension column was monitored continuously with an in-line pH meter. Protein fractions were eluted based on their pI, measured for absorbance at 280 nm, and collected in a 96 deep-well plate by a fraction collector according to predetermined pH decrements of 0.3 pH units during the pH gradient, or in 1.5 mL volumes before and after the pH gradient. Therefore, the chromatofocusing separation took approximately 220 min and a total of 30 first-dimension fractions were collected over this period.

2.15.2.3 High-performance reverse-phase chromatography

High performance reverse-phase chromatography was carried out on an HPRP 2D column (4.6 x 33 mm; 1.5 mm particle size) (Beckman Coulter, Inc., Fullerton, CA) which was pre-equilibrated with 0.1% (v/v) trifluoroacetic acid (TFA) in water. The mobile phase consisted of 0.1% (v/v) TFA in water (A) and 0.08% (v/v) TFA in acetonitrile (ACN) (B). Separation was performed at 0.75 mL/min with an increasing gradient of B. During the first 2 min, 100% of A was pumped into the column; in the next 35 min the gradient was created in the column by switching the flow from 0 to 100% B; this was followed by 100% B for 4 min and 100% A for 9 min. The first-dimension fractions were first analyzed by injecting 200 μL of each on to the second-dimension column to determine the fractions that were necessary for mass spectrometry analysis. During this mapping mode, second dimension fractions were not collected. An aliquot of 500 μL of each of the selected first-dimension fractions was then re-run and fractions collected in a 96-well plate by an automated fraction collector at intervals of 15 sec between 4-24 min. Collected second-dimension fractions were stored at -80^{o}C for subsequent mass spectrometry analysis. The column temperature was maintained at 50^{o}C and absorbance of the column effluent was monitored by a second high performance UV detector at 214 nm.

2.15.2.4 Data analysis

Multiple chromatography traces from the second dimension separation were converted into 2D protein expression maps by importation into the ProteoVue software program. Differential protein expression experiments of control and treatment samples were carried out by comparing two ProteoVue 2D protein expression maps with the assistance of the DeltaVue software package. DeltaVue and ProteoVue are part of the 32 Karat software that controls the ProteomeLab^{TM} PF2D system. The analysis was based on comparing the peak height of proteins and peptides that elute in the same pH fraction in the first dimension and at the same time in the second dimension.

2.15.3 Peptide preparation for mass spectrometry analysis

2.15.3.1 In-gel trypsin digestion

Protein spots of interest were manually excised and the gel plugs transferred into 96-well microtitre plates, sealed, and stored at -20°C until further processing. They were destained twice using a solution of 50 mM ammonium bicarbonate (NH_4HCO_3) containing 50% (v/v) ACN followed by dehydration using ACN. Proteins were reduced for 30 minutes in a solution containing 10 mM DTT in 100 mM NH_4HCO_3 and alkylated with 55 mM iodoacetamide in 100 mM NH_4HCO_3 for 20 minutes. The excess solution was removed and the gel plugs washed in 50 mM NH_4HCO_3 containing 50% (v/v) ACN, then dehydrated using ACN and heated to 37°C. Proteins were digested with trypsin (Promega, UK) at a concentration of 6 ng/μL in 100 mM NH_4HCO_3 for 3 hours at 37°C. The resulting peptides were extracted using aqueous 1% (v/v) formic acid containing 2% (v/v) ACN.

2.15.3.2 In-solution trypsin digestion

An aliquot of 175 μL of each PF2D fraction was transferred to a 96-well microtitre plate. Each plate was flash frozen on dry ice for at least 10 min and transferred to a desiccating chamber. A vacuum was applied until the samples were completely dried. Each sample was rehydrated with 10 μL of 100 mM NH_4HCO_3 buffer (Sigma-Aldrich, UK) followed by reduction with 15 μL of 10 mM DTT for 30 minutes and subsequently alkylation with 15 μL of 55 mM iodoacetamide for 20 minutes. A 12.5 μL aliquot of 6 ng μL^{-1} trypsin (Promega, UK) was added to each sample and allowed to incubate at 37°C for 3 hours. The resulting peptides were acidified with 20 μL of 1% (v/v) formic acid and, if necessary, stored at 80°C prior to analysis by mass spectrometry.

2.15.4 Mass spectrometry analysis

2.15.4.1 Analysis using matrix-assisted laser desorption/ ionisation (MALDI) mass spectrometry

The tryptic peptides obtained by in-gel tryptic digestion were characterized by means of matrix-assisted laser desorption/ ionisation (MALDI) mass spectrometry utilizing an Applied Biosystems 4800 MALDI TOF/TOF analyzer (Applied Biosystems, Foster City, CA, USA). The samples were mixed in a 1:1 ratio with α-Cyano-4-hydroxycinnamic acid (Fluka) matrix dissolved in 50% GC grade acetonitrile (Burdick and Jackson, Muskegon) containing 0.1% trifluoroacetic acid (Sigma St Louis) and 0.5 μL spotted on the source plate. Data was acquired in reflectron positive mode with internal calibration using bovine trypsin autolytic fragments calibration and the scan range set to m/z 800-4000. Spectra were recorded at 50 shots/sub spectrum with a total of 1000 spectra. The source voltage was set to 20 kV with the grid voltage at 16 kV. Delayed extraction time was to 400 ns. Data processing was performed using the local MASCOT server.

2.15.4.2 Analysis using in-line liquid chromatography and electrospray ionization mass spectrometry

The 96-well microtitre plate containing tryptic peptides obtained by in-solution trypsin digestion was transferred to a Micromass modular CapLC and autosampler system. A 6.4 μL aliquot of extract was mixed with 13.6 μL of 0.1% (v/v) formic acid and loaded onto a 0.5 cm LC Packings C18 5 μm 100Å 300 μm i.d μ-precolumn cartridge. Flushing the column with 0.1% (v/v) formic acid desalted the bound peptides before a linear gradient of solution B at a flow rate of 200 nLmin^{-1} eluted the peptides for further resolution on a 15 cm LC Packings C18 5 μm 5Å 75 μm i.d. PepMap analytical column (Dionex, USA). Conditions are described in Table 2.5. Solution A consists of 95% (v/v) water, 4.9% (v/v) acetonitrile, 0.1% (v/v) formic acid while solution B of 4.9% water, 95% (v/v) acetonitrile, 0.1% (v/v) formic acid.

Table 2.5: Column gradient conditions

Time (minutes)	%A	%B
0.1	95	5
3	95	5
31	55	45
35	20	80
37	20	80
38	95	5
47	95	5

The eluted peptides were analyzed on a Micromass QTof Global Ultima mass spectrometer (Waters MS Technologies, Manchester, UK) fitted with a nano-LC sprayer with an applied capillary voltage of 3.5 kV. The instrument was calibrated against a collisionally induced decomposition (CID) spectrum of the doubly charged precursor ion of [glu^1]-fibrinopeptide B (GFP). A calibration was accepted when the error obtained on all subsequent acquisitions was < 20 ppm. Sensitivity is assessed by the detection of a 500 fmol injection of GFP, with a base peak signal:noise ratio of $> 50{:}1$ on the doubly charged ion. Both the sensitivity and calibration of the MS instrument were checked at regular intervals during the analysis as well as the chromatographic resolution of the GFP peak.

Protein identification

The instrument was operated in data dependent acquisition (DDA) mode over the mass/charge (m/z) range of 50-2000. During the DDA analysis, both MS and tandem mass spectrometry (CID) was performed on the most intense peptides as they elute from the column. The uninterpreted MS/MS data were processed using the Micromass ProteinLynx Global Server software package (smoothed, background subtracted, centred and deisotoped), which then searched the MS/MS spectra against an appropriate database using the Micromass Global Server 2.2 search engine. Search parameters specify up to two missed cleavage sites, a 100 ppm tolerance against the database-generated theoretical peptide and product ion masses and a minimum of 1 matched peptide. A list of the twenty highest scoring entries was produced and

each suggested protein identification was confirmed or rejected by a comparison of the theoretical sequence with observed MS/MS data. When the database search was unsuccessful, the MS/MS spectra were interpreted in order to obtain amino acid sequence tags. Spectra were interpreted automatically using the Peaks Studio 4.5 software package (Disinformation Solutions Inc.) then manually verified and/or improved by manual intervention and probabilistic peptide sequences suggested.

Chapter 3

Transcriptomic Analysis of *Arabidopsis thaliana* - *Botrytis cinerea* Interaction

3.1 Rationale

Although plants are continuously exposed to a wide range of abiotic (e.g. drought, high salinity, low and high temperatures) and biotic (e.g. viruses, bacteria and fungi) stresses, they effectively respond and adapt to these stresses. Plants respond through employing various biochemical and physiological processes which include production of hormones (e.g. ABA), signalling compounds (e.g. ET, JA and SA), antimicrobial compounds, antioxidants and many others (Kettner and Dörffling, 1995; Wan *et al.*, 2002). These processes are often activated by products of genes that are transcriptionally induced following plant exposure to any of the above mentioned stresses. Many studies have demonstrated that large scale changes in transcription take place in response to stress (van der Biezen *et al.*, 2000; Mahalingam and Fedoroff, 2003). However, these changes are more pronounced in tolerant than susceptible plants; for instance, Evers *et al.* (2003) compared differential gene expression in two potato lines differing in resistance to the late blight fungus, *P. infestans* and found that most of the differentially expressed genes were expressed in the resistant line. Such information is very vital in developing plants that can tolerate or resist abiotic and biotic stresses.

Therefore, the first step in identifying genes for biotechnological applications, is establishing the expression profiles of different genes after exposing plants to specific stresses. This can be achieved by comparing the abundance of mRNA transcripts in treated and control plants. There are various methods for detecting as well as quantifying the abundance of mRNA transcripts. Methods such as northern, dot and slot blots (Mauvieux *et al.*, 1998), nuclease protection assays (e.g. ribonuclease protection and S1 nuclease assays) (Thompson and Sommercorn, 1992), quantitative and semi-quantitative polymerase chain reaction (PCR) (Wong and Medrano, 2005) can be used but are suited for analyzing single specific or very few transcripts. Methods that can detect a significant number of transcripts include *in situ* hybridization (Hofler, 1990), subtractive hybridization (Hara *et al.*, 1991), suppressive subtractive hybridization (Diatchenko *et al.*, 1996), serial analysis of gene expression (SAGE) (Velculescu *et al.*, 1995), arbitrary primed PCR (Welsh *et al.*, 1992), differential display PCR (Liang and Pardee, 1992) and cDNA amplified fragment length polymorphism (cDNA-AFLP) (Bachem *et al.*, 1998; van der Biezen

et al., 2000) however; these methods are biased towards abundant transcripts.

A recent technology that has revolutionalized transcriptome studies referred to as microarrays, offers more advantages such as determination of expression levels of tens of thousands of different mRNA transcripts and is sensitive to low abundant transcripts (Alba *et al.*, 2004). The use of such a technology which can profile gene expression on a global level, provides enormous information that can be used to develop a more complete understanding of gene function, regulation and interactions. For instance, genes regulated by similar mechanisms are normally grouped in the same cluster (Maleck *et al.*, 2000). Microarrays can be defined as a collection of microscopic DNA spots, commonly representing single genes, arrayed on a solid surface by covalent attachment to chemically suitable matrices (Venkatasubbarao, 2004). All microarray systems have three components; the array containing the target (spots), probes that are hybridized with the target and a detection system to quantify hybridization (Cheung *et al.*, 1999). The terms probe and target are normally used interchangeably however, in this study the target refers to the DNA spotted on the array or the immobile substrate while the probe refers to the DNA hybridized to the array or the mobile substrate.

Microarrays are mainly of two types, cDNA and oligonucleotide microarrays. In cDNA microarrays, the targets are PCR products (400-1000 base pairs) derived from either amplification of clones from a cDNA library or bacterial artificial chromosome (BAC) clones (Rensink and Buell, 2005). Examples of cDNA microarrays include the CATMA (the Complete Arabidopsis Transcriptome MicroArray) arrays (Allemeersch *et al.*, 2005). In oligonucleotide microarrays, the targets are made of oligonucleotides which are either spotted on to the array substrate as in the case of oligonucleotide spotted arrays (50-70 base pairs) or synthesized *in situ* as in the case of on-slide synthesized arrays (25 base pairs) (Rensink and Buell, 2005). Examples of on-slide synthesized arrays include those made by Affymetrix (http://www.affymetrix.com), Agilent (http://www.agilent.com) and Nimblegen (http://www.nimblegen.com) while in this study, we used oligonucleotide spotted arrays from Arizona University (http://www.ag.arizona.edu/microarray) (Rensink and Buell, 2005).

A typical microarray experiment starts with extraction of RNA from two samples (control and test sample) which is subsequently converted to cDNA and labeled with different fluorescent dyes like Cyanine dye Fluors (Cyanine 3 and Cyanine 5). Cyanine dyes are preferred because of their stability, sensitivity and resistance to photo-bleaching (Waggoner, 2006). Equal amounts of labeled cDNA from each sample are combined and spread on top of the microarray slides to hybridize with the targets. After hybridization, the fluorescent signals corresponding to the two dyes are measured and the resulting images analyzed to obtain numerical expression data. The main assumption underlying microarray experiments is that the intensity of the hybridization signal for any sequence is proportional to the amount of mRNA corresponding to that sequence in the original mRNA sample (Moody, 2001). Relative expression of each sequence represented on the microarray is therefore evaluated by comparing hybridization intensity signals generated by different experimental samples. A higher signal in the control than the test sample suggests a down-regulation, while the opposite an up-regulation.

Microarrays have been extensively used to monitor expression profiles of genes induced by various stresses in different organisms including plants and have resulted in the identification of many stress responsive genes. Rabbani *et al.* (2003) used a cDNA microarray containing 1,700 independent rice cDNAs and identified 36, 62, 57, and 43 genes induced by cold, drought, high salinity, and abscisic acid. Schenk *et al.* (2003) studied the incompatible interaction between *Arabidopsis* and *A. brassicicola* using a cDNA microarray containing 2,375 genes and identified 705 genes that responded to this fungal pathogen. Studies involving compatible interactions have also revealed changes in expression profiles of various host genes following infection. For instance, Lopez *et al.* (2005) identified 199 that were differentially regulated after infecting cassava with the bacterium *Xanthomonas axonopodis* pv *manihotis*. Microarrays have also been used to study expression patterns of pathogens genes during infection, a procedure that is providing valuable information for developing disease control strategies (Moy *et al.*, 2004). We therefore took advantage of the existence of this technology to study the molecular interaction between the model cruciferous plant *A. thaliana* and the destructive necrotrophic deuteromycete *B. cinerea* with the view of identifying genes that may play a role in resistance.

3.2 Results

3.2.1 Selection of time points for transcriptome analysis of *Arabidopsis* leaves infected with *B. cinerea*

The main of aim these experiments was to determine the method of inoculation and the time points to use in the two microarray experiments.

3.2.1.1 Determination of the method of inoculation

Two methods of inoculation were tested to identify one which gives the most reproducible infections. In the first method, *Arabidopsis* leaves were detached from whole plants and placed on trays containing 0.8% (w/v) agar. Depending on the size of the leaf, 8-10 drops of 10 μL spore suspension (500,000 spores mL^{-1}) were placed on top of each leaf and inoculations covered. In the second method, whole plants were sprayed with spore suspension of the same concentration until the droplets ran off and left under growth room conditions. Distinct necrotic lesions were observed with drop inoculations of detached leaves after 24 hpi. Although whole plants were sprayed, not all leaves were infected while those that were infected, lesions were only observed by 48 hrs (Fig. 3.1).

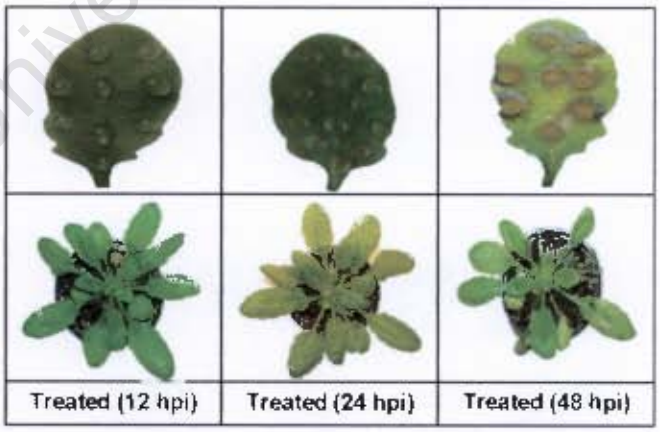


Figure 3.1: Four week old *Arabidopsis* (Col-0) detached leaves and whole plants were inoculated with *B. cinerea* over a time course. More reproducible infections were obtained when detached leaves were inoculated with drops than when whole plants were sprayed with inoculum.

3.2.1.2 Detection of camalexin in infected *Arabidopsis* leaves

Phytoalexins are low molecular weight antimicrobial metabolites produced in plants in response to pathogen and elicitor treatment (Morrissey and Osbourn, 1999). Camalexin (3-thiazol-2'-yl-indole) is the main phytoalexin produced in *Arabidopsis* (Tsuji *et al.*, 1992) and has been shown to be induced following *B. cinerea* infection (Govrin and Levine, 2002; Ferrari *et al.*, 2003). To determine when camalexin starts to accumulate after infection, *Arabidopsis* leaves were inoculated with *B. cinerea*. Mock treated and infected leaves were harvested after every 2 hrs for a period of 24 hrs. Camalexin extraction was performed for each time point and separated using thin layer chromatography. It was visualized under a long wavelength UV filter as a blue fluorescent compound. Camalexin accumulation was observed as early as 8 hpi before any visible lesions (Fig. 3.2).

Figure 3.2: Four week old *Arabidopsis* (Col-0) leaves were inoculated with *B. cinerea* over a time course, camalexin was extracted, separated using thin layer chromatography and visualized under UV light as a blue fluorescent compound. Although camalexin was extracted from infected *Arabidopsis* leaves harvested after every 2 hrs, it was only visualized in leaf tissue harvested after 8 hrs.

3.2.1.3 Detection of H_2O_2 accumulation in infected *Arabidopsis* leaves

The oxidative burst is one of the earliest defence responses following pathogen recognition. Although it is effective against biotrophic pathogens, it has been shown to promote host colonization by necrotrophs (Govrin and Levine, 2000). A number of studies have provided evidence for accumulation of AOS in tissues infected by *B. cinerea*; some of these AOS have been shown to be of pathogen origin (Muckenschnabel *et al.*, 2001, 2002, 2003). Accumulation of AOS especially H_2O_2 can be detected by *in situ* histochemical staining of infected leaves using 3,3-diaminobenzidine

(DAB) (Thordal-Christensen *et al.*, 1997). To determine how soon H_2O_2 accumulation could be detected, *Arabidopsis* leaves were inoculated with *B. cinerea*, mock treated and infected tissue were then harvested after every 2 hrs for period of 24 hrs and immediately stained with DAB. Presence of H_2O_2 was observed as a reddish-brown colouration around the site of inoculation by 8 hpi (Fig. 3.3).

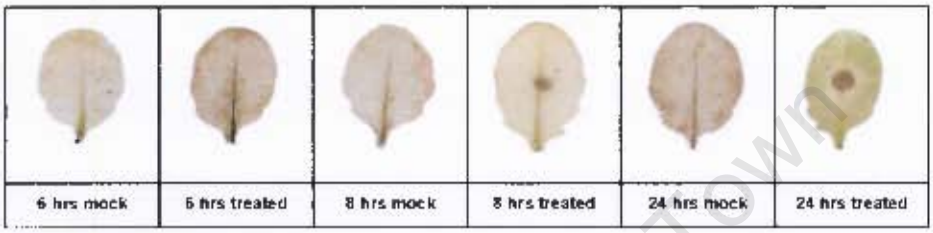


Figure 3.3: Four week old *Arabidopsis* (Col-0) leaves were inoculated with *B. cinerea*. Mock-treated and infected leaves were harvested after every 2 hrs and immediately stained for H_2O_2 accumulation using DAB stain. The experiment was carried out for a period of 24 hrs. H_2O_2 accumulation was observed as a reddish-brown colouration around the lesion only in leaves infected and harvested after 8 hrs.

3.2.1.4 Detection of *OXI1::GUS* expression in infected transgenic *Arabidopsis* lines

The *Oxidative Signal-Inducible1* (*OXI1*), is an *Arabidopsis* gene (*At3g25250*) encoding a serine/threonine kinase that was shown to be rapidly induced (5 min) by H_2O_2 as well as other stimuli that generate H_2O_2 (e.g wounding and pathogen infection) (Rentel *et al.*, 2004). Due to the fact that H_2O_2 is generated following infection by *B. cinerea*, *OXI::GUS* was used to determine the earliest time of its expression which could also indicate the response of the plant to this pathogen. *Arabidopsis* leaves carrying the *OXI1::GUS* gene construct were inoculated with *B. cinerea*. Mock treated and infected leaves were harvested after every 2 hrs for a period of 24 hrs and immediately stained for GUS expression. GUS activity driven by *OXI1* expression was observed to be induced as early as 12 hpi (Fig. 3.4).

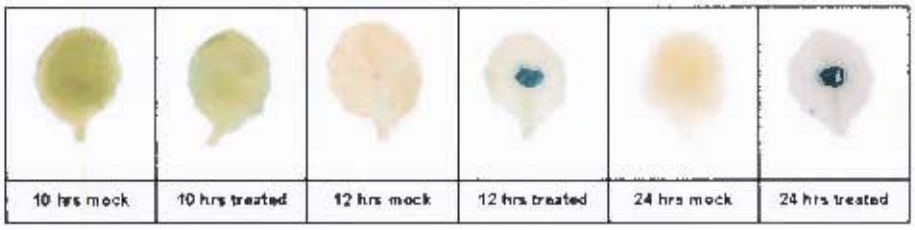


Figure 3.4: Four week old transgenic *Arabidopsis* leaves containing the *OXI1::GUS* gene construct were inoculated with *B. cinerea*. The experiment was carried out for a period of 24 hrs and mock-treated and infected leaves were harvested after every 2 hrs and immediately stained for GUS expression. GUS activity driven by *OXI1* expression was only observed in *Arabidopsis* leaves infected and harvested after 12 hrs.

Based on the results from the above experiments, drop inoculation of detached leaves was chosen as the method of inoculation because it gave the most reproducible infections over a time course. Because *B. cinerea* spores need to germinate on the leaf surface, then penetrate the leaf and affect sufficient cells in the leaf that gene expression and biochemical changes can be detected, the 12 hr time point was chosen as the early time point since the first biochemical and gene expression changes occurred by this time. The 24 hr time point was chosen as the late time point.

3.2.2 Expression profiling of infected *Arabidopsis* leaves

3.2.2.1 Temporal gene expression

The ability of a specific cultivar to resist or tolerate infection by a specific pathogen depends on how soon that cultivar detects invasion and timely deployment of the necessary defence mechanisms. These early responses by plants to invasions by pathogens can be observed at the transcription level (Scheideler *et al.*, 2001; van Wees *et al.*, 2003). This experiment was therefore conducted to determine the changes in transcript abundance that occur with the view of identifying early as well as late responsive genes following infection of *Arabidopsis* leaves with *B. cinerea*. Four week old *Arabidopsis* leaves were inoculated with *B. cinerea* and harvested after 12 and 24 hpi. This experiment was replicated (biological) five times. In the first replicate, infected cDNA was labeled with Cy3 while mock treated cDNA was labeled with

Cy5. In the last four replicates, the dyes were swapped with the infected cDNA being labeled with Cy5 and the mock treated cDNA with Cy3 (Table. 2.2).

3.2.2.2 Spatial gene expression

This experiment was performed to examine spatial changes in gene expression following infection of *Arabidopsis* leaves with *B. cinerea* with the view of identifying genes specifically expressed close to and away from the lesion. The reasons for this experiment were based on studies carried out recently by Ferrari *et al.* (2003) and Kliebenstein *et al.* (2005). Ferrari *et al.* (2003) demonstrated that *PR1* expression was induced locally at the edge of the developing *B. cinerea* lesion which implicated SA in regulation of local resistance of *B. cinerea* in *Arabidopsis*. Kliebenstein *et al.* (2005) found high concentrations of camalexin in areas around the lesion while that of other secondary metabolites such as the glucosinolates, flavonols and sinapates decreased closer to the lesion and almost disappeared in tissues directly proximal to the lesion. They postulated that a signal produced at the edge of the lesion possibly by the host or the pathogen regulates metabolite levels and diffuses outwards towards uninfected tissue.

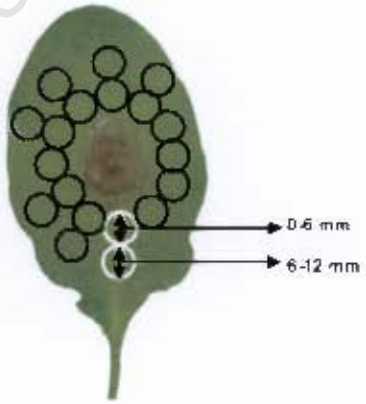


Figure 3.5: Four week old *Arabidopsis* leaves were inoculated with a single drop of spore suspension in the middle of each leaf. After 48 hrs, the infected part (lesion) of the leaves was removed and tissue harvested close to (0-6 mm) and away from (6-12 mm) the lesion using a cock borer of 6 mm in diameter.

This experiment examined if this spatial regulation could be reflected in expression profiles of different genes. Performing the spatial experiment may also highlight additional genes only differentially expressed in a small area which are missed when taking the whole leaf. In this experiment, four week old *Arabidopsis* leaves were inoculated with a single drop of *B. cinerea* spore suspension in the middle of the leaf and left on the bench-top for 48 hrs. A similar treatment was made for control leaves but with half-strength grape juice without *B. cinerea* spores. Using a cock borer of 6 mm in diameter, leaf disks were cut from the edge of the lesion (0-6 mm) and exactly after the first cut (6-12 mm) (Fig. 3.5). This experiment was conducted in three replicates however, the third replicate was a dye swap (Table. 2.2). Biological comparisons were made between mock treated leaf disks and disks (0-6 and 6-12 mm) from inoculated leaves.

3.2.2.3 Normalization of microarray data

Following data acquisition, all data from the two experiments was normalized. The purpose of normalization is to identify and remove as much technical variation as possible so that the expression levels that are observed can be attributed to the biological differences between the RNA samples or the printed probes (Sartor *et al.*, 2003). Technical variation is caused by a number of factors which include sample homogeneity, RNA quality, differences in labeling efficiencies or scanning properties of the dyes, differences in scanning parameters, and non-uniformity of hybridization (Quackenbush, 2001; Hatfield *et al.*, 2003).

Differences in labeling efficiencies can be attributed to the differences in physical properties of the dyes such as heat and light sensitivity, relative half-life and others (Yang *et al.*, 2001). For instance higher intensities are normally recorded for Cy3 than Cy5. One of the reasons that possibly causes this is related to the wavelength. The energy of a photon is inversely proportional to its wavelength as a result photons with short wavelength like those of Cy3 (λ = 550 nm) easily absorb and re-emit light energy than photons of longer wavelength like those of Cy5 (λ = 650 nm). Quenching is another factor that may affect the intensities of the two dyes. It is a phenomenon where dyes in close proximity reabsorb light from each other causing decreased fluorescent signals (Ramdas *et al.*, 2001). All these causes

of technical variation result in an imbalance of the two channels which should be corrected by normalizing one channel against the other so as to obtain an unbiased representation of relative expression.

A number of approaches for normalizing dual channel microarray data are available. Examples include total intensity, linear regression, iterative log (ratio) mean centering, rank invariant methods, and ratio statistics (Quackenbush, 2001). However, all these approaches do not account for the systematic bias that is characteristic of microarray data. $Log_2(R/G)$ values have been shown to have a systematic dependence on intensity, which commonly appears as a deviation from zero especially for low-intensity spots (Yang *et al.*, 2001; Quackenbush, 2002). These intensity-dependent patterns can be visualized by plotting $log_2(R/G)$ values for each spot as a function of $log_2(R^*G)$ product intensities (Fig. 3.6). The $log_2(R/G)$ is also referred to as the M value, a mnemonic for *minus* since it can be written as (log_2R - log_2G) while $log_2(R^*G)$ is referred to as the A value, a mnemonic for *add* since it can be written as (log_2R + log_2G)/2 (Smyth and Speed, 2003). The M value represents the log differential ratio for each spot and A value the average intensity of each spot. This plot is either referred to as an RI (ratio-intensity) or an MA plot (Quackenbush, 2002).

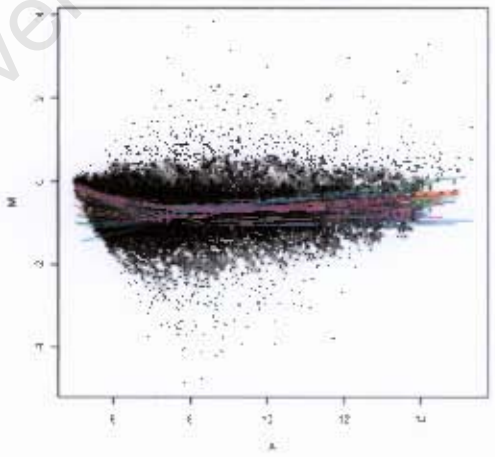


Figure 3.6: A plot of log of ratios (M) against log of average intensities (A) for one of the slides before normalization. The different lines represent lowess curves for each print-tip group in this slide. An intensity dependent pattern is clearly observable for each print-tip group which is good evidence of the need for print-tip normalization.

Removing intensity-dependent patterns can be achieved by fitting a smooth curve through the points. One example of such a curve is a locally weighted linear regression scatter-plot smooth (lowess or loess) curve. It is constructed by performing a series of local regressions at each point in the scatter plot (Yang *et al.*, 2002). The local regressions are based on 40% of the spots which are closest in terms of A value to the spot being predicted and are estimated by using the re-descending M estimation with Turkey's biweight function. By carrying out local linear regressions as a function of the $\log_2$(intensity) and subtracting the calculated best-fit average $\log_2$(ratio) from the experimentally observed ratio for each data point, loess normalization corrects for these systematic deviations (Smyth and Speed, 2003). Loess may be applied to the entire data set (global) or some physical subgroups of data on the array (locally) such as print-tip groups (Yang *et al.*, 2002). Print-tip groups also referred to as pen groups or sub-grids are a group of array elements deposited by the same printing pin during array fabrication (Causton *et al.*, 2003).

Local loess normalization is responsible for removing systematic differences that may exist between arrays or sub-grids on the same array. These systematic differences arise as a result of many factors which include inconsistencies among the spotting pins, variability in the slide surface, slight local differences in hybridization conditions across the array and hybridization differences especially when the probe does not spread evenly along the slide. Differences caused by the printing pin may be attributed to differences in length and opening of the tip or deformation after many hours of printing. These differences result in variations in log ratios within each print-tip group. Based on this background, print-tip loess normalization was used. It is the default method of normalization in the diagnosis and normalization for microarray data analysis tool (DNMAD) (Vaquerizas *et al.*, 2004). DNMAD is part of gene expression pattern analysis suite (GEPAS), a web-based resource for microarray gene expression data analysis (Herrero *et al.*, 2003).

Print-tip loess performed in DNMAD, uses the majority of the genes for normalization. This is because biological comparisons made on microarrays are normally very specific in nature and only a small proportion of genes is expected to be differentially expressed (Yang *et al.*, 2001). The remaining genes are expected to have a constant expression and therefore can be used as indicators of relative

intensities of the two dyes. Many studies carried out using microarrays support this assumption. For example, Schenk *et al.* (2000) used a targeted microarray containing 2.375 *Arabidopsis* genes and identified only 705 genes which responded to infection by *A. brassicicola*. Desikan and associates (2001) used a cDNA microarray representing about 30% of the *Arabidopsis* genome and identified only 175 non-redundant expressed sequence tags (ESTs) that were regulated by H_2O_2. This assumption may also hold true for these experiments because full genome slides were used and only a fraction of the *Arabidopsis* genome is expected to change in expression. Another condition of using print-tip in DNMAD is that the number of points to be normalized must be large in each print-tip group. In this study, slides with a 4 x 12 main grid and 25 x 26 sub grid (print-tip group) which is 650 points for each print-tip group were used which meets this condition.

GenePix result (GPR) files obtained after image analysis were uploaded into DNMAD for normalization. Negative flags were used however, no background subtraction was done. This is because background subtraction has been shown to increase the variance of Cy5/Cy3 ratios especially for low expressed genes (Sartor *et al.*, 2003). However, the half option of background correction in DNMAD was selected. In this option intensities less than 0.5 after subtracting the background are reset to 0.5. This minimizes the variance of Cy5/Cy3 ratios for the low expressed genes. The output from normalization was pre-processed and under this the data set was log transformed to the base 2. Base 2 was used because it provides easy interpretation of data when viewing fold changes. For instance, M = 0 represents equal expression since a log of 1 is 0, M = 1 represents a 2 fold change (up-regulation) in mRNA samples while M = -1 represents a 2 fold change but down-regulation (Quackenbush, 2001). Figure 3.7 shows the MA plot after normalization.

There are two main reasons why microarray data should be log transformed; 1) the distribution of raw data is skewed to the far left because the majority of genes are transcribed at low levels hence log transformation of data approximates a normal distribution. This is very important especially if parametric tests are to be used in downstream data analyses; 2) random error in microarray measurements increase with higher signal intensities hence log transforming helps in normalizing these random errors (Sartor *et al.*, 2003). After normalization, M and A values for

each slide for each gene spot were downloaded. M values of slides from the first and third replicates of the temporal and spatial experiments respectively were multiplied by -1 because they were dye swaps.

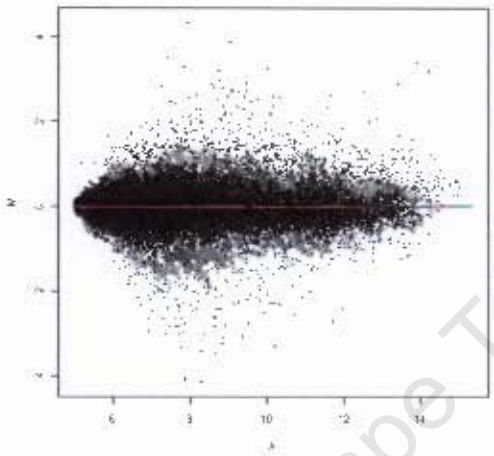
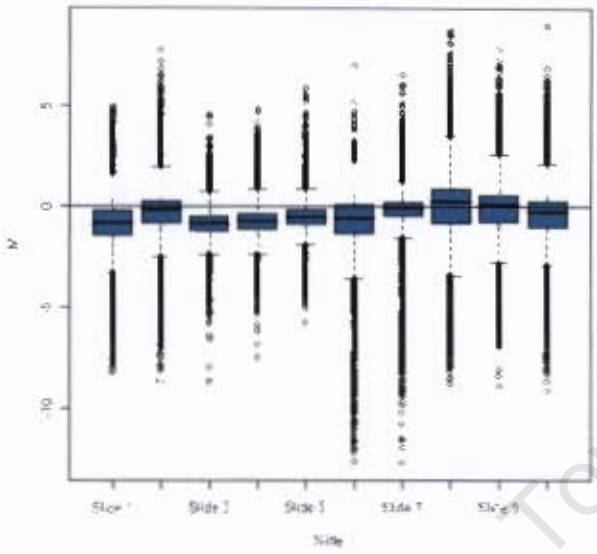
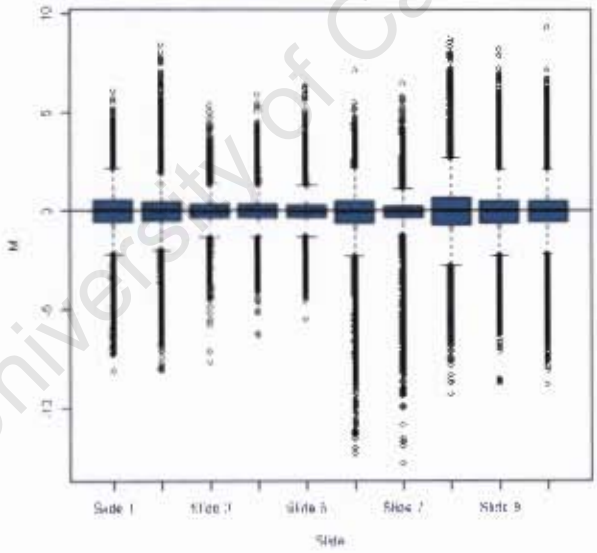


Figure 3.7: A plot of log of ratios (M) against log of average intensities (A) for one of the slides after normalization. The different lines represent lowess curves for each print-tip group in this slide. An intensity dependent pattern is clearly observable for each print-tip group which is good evidence of the need for print-tip normalization.

Box plots are also used to visualize data sets and provide evidence for the need for global (Fig. 3.8) and print-tip loess (Fig. 3.9) normalization. They provide five important descriptive statistics, the minimum, lower quartile, median, upper quartile and maximum values (Keller, 2005). Lines and points extending outside the boxes are called whiskers and outliers respectively. Outliers represent errors within data sets or unusual observations that require investigation and in this experiment, they could represent differentially expressed genes. The underlying assumption is that most genes should not change hence M = 0 ($\log_2 1$). It is observable that the median of the data in individual box plots lies below but aligned along M = 0 after normalization. This implies M values are negative and negative M values are obtained only when the intensity of the green channel is higher than the red channel giving a ratio less than one hence a negative log. This confirms the earlier point that Cy5 intensities are usually less than those of Cy3. Normalization both global and print-tip loess helped to correct for noise created by technical variation.

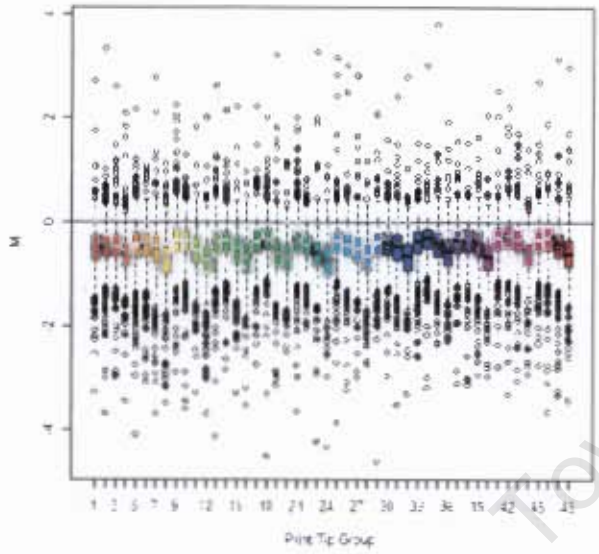


A

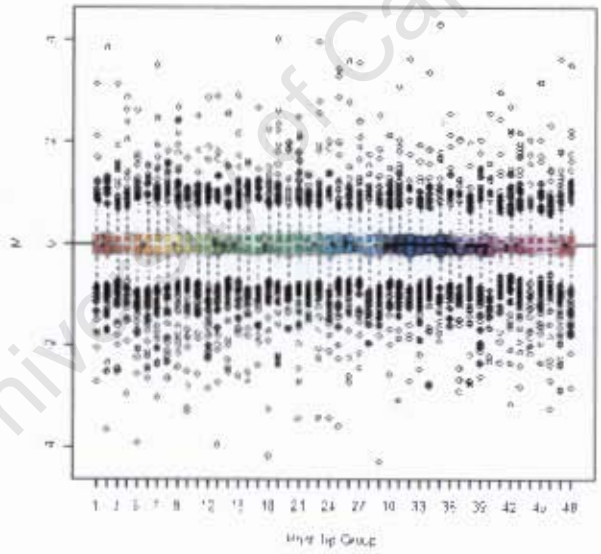


B

Figure 3.8: A plot of the distribution of log of ratios (M values) within the 10 microarray slides used in the time-course microarray experiment before (A) and after (B) normalization. Variability and deviation from M = 0 of log ratios within each slide as demonstrated by the median of each boxplot before normalization is good evidence of the need for global loess normalization.



A



B

Figure 3.9: A plot of the distribution of log of ratios (M values) within each print-tip group for one of the slides before (A) and after (B) normalization. The 48 boxplots represent the 48 print-tip groups in this slide. Variability and deviation from M = 0 of log ratios within each print-tip group as demonstrated by the median of each boxplot before normalization is good evidence of the need for print-tip normalization.

3.2.2.4 Determination of differentially expressed genes

Besides discovering patterns of expression (Eisen *et al.*, 1998; Quackenbush, 2001; Thibaud-Nissen *et al.*, 2003), the other main objective of microarray studies is the identification of genes whose expression levels in the treated sample are significantly different from the control. In many studies, a threshold often a two fold change in up- or down-regulation has been used to determine genes that are differentially expressed (Schena *et al.*, 1995; Schenk *et al.*, 2000; Scheideler *et al.*, 2001; AbuQamar *et al.*, 2006). However, a two fold change does not have any statistical meaning neither does it present any biological significance (Hatfield *et al.*, 2003). The disadvantage with this method is that it does not take into account the variability in expression of each gene and as a result, genes with large variances have a good chance of giving large average log ratios even when they are not differentially expressed. This can be improved by performing a statistical test on the log ratios (Hatfield *et al.*, 2003). Since we are looking at two conditions (treated and control), a t-test would be the best option. This provides a t-statistic which can then be used as a basis for selecting differentially expressed genes. Because the data was log transformed, it therefore conforms to the assumption underlying this test which is normality and equal variances (Keller, 2005).

The t-statistic is the ratio of the difference between two means or average and the standard deviation. The advantage with this test is that it introduces some conservative protection against outlier log ratios as these will give rise to large standard deviations preventing such genes from being spuriously identified as differentially expressed. However, this test suffers from two shortcomings; first, it can identify genes with small sample variances as differentially expressed even when they are not; second, it does not take into account the issue of multiple testing. This is because a statistical test for differential expression is conducted for each gene at a fixed gene-wise significance level. If this level is fixed for instance at $P \leq 0.05$, it means one gene in every 20 that are actually not differentially expressed will show a P-value below 0.05 by chance. Because gene expression profiling is done on a genome-wide level, this translates into a large number of false positives.

Two approaches that can be used in identifying differentially expressed genes while controlling for false discoveries are family-wise error rate (FWER) and false discovery rate (FDR). FWER is the probability that the selected set of genes contains at least one false positive while FDR is the expected proportion of false positives among the rejected hypotheses (Slonim, 2002). Procedures that control the FWER include the Bonferroni correction and stepwise P-value adjustment of Westfall and Young (Westfall and Young, 1993) however, these procedures are often too stringent leading to high rate of false-negatives (Nadon and Shoemaker, 2002). FDR control is preferred because it is less conservative and can be used at a desired significant level or with permutation methods such as significant analysis of microarrays (SAM) (Tusher *et al.*, 2001).

SAM identifies genes with statistically significant changes in expression by assimilating a set of gene-specific t-tests. The expression data for each gene are permutated and a test statistic d is computed for both the original and the permutated data for each gene. Genes with d scores greater than a threshold are deemed potentially significant. The percentage of such genes identified by chance is the FDR. To estimate the FDR, nonsense genes are identified by analyzing permutations of the measurements. The threshold can be adjusted to identify smaller or larger sets of genes, and FDRs are calculated for each set. Because the aim was to identify genes significantly differentially expressed between the treated sample and the control, a one class t-test of SAM was therefore conducted with a 5% FDR. SAM is available in TMEV, a free open-source software from TM4 (Saeed *et al.*, 2003). The mean value to be tested against was set to zero (no change in expression).

The number of genes identified by SAM to be significantly differentially regulated in the temporal experiment were 2,026 and 6,306 genes at 12 and 24 hpi respectively. Considering a cut-off of log of 1 or 2 fold change, 976 genes were selected at 12 hpi of which 253 were up-regulated (Appendix. A.1) and 723 were down-regulated (Appendix. A.2) while 2,606 genes were selected at 24 hpi of which 1,109 were up-regulated (Appendix. A.3) and 1,497 down-regulated (Appendix. A.4). Genes up- and down-regulated in the two time points were divided into three categories, category 1 consisted of genes up- and down-regulated only after 12 hrs, category 2 consisted of genes up- and down-regulated after 12 hrs but their abun-

dance significantly increased (up-regulated) or reduced (down-regulated) by 24 hrs while category 3 consisted of genes whose abundance increased and reduced significantly by 24 hrs. For the up-regulated genes, category 1, 2 and 3 consisted of 67, 186, and 923 genes respectively (Fig. 3.10A) while for the down-regulated genes, category 1, 2 and 3 consisted of 134, 589, and 903 genes respectively (Fig. 3.10B).

Examples of up-regulated genes in category 1 included disease resistance protein, disease resistance protein, F-box family protein, pectin methylesterase inhibitor, lectin family protein (*At2g39810*), protease inhibitor, protein kinases, zinc finger proteins and WRKY family transcription factor (*At2g25000*); category 2 consisted of genes such as catalase 3 (*SEN2*), ACC oxidase, ACC synthase, ABC transporters, anthranilate synthase, chitinase, GSTs, *PR4*, patatin, peroxidase, *PGIP1*, proteinase inhibitors, *SEN1*, tryptophan synthase and WRKY transcription factor (*At1g62300*) while examples of category 3 included ABC transporter family proteins, carbonic anhydrases, GSTs, MYB transcription factors, nitrilases, *PDF1.2a*, peroxidases, *PGIP2*, protein kinases, protease inhibitors, *PR1*, terpene synthases, zinc finger proteins and UDP-glucosyl transferases.

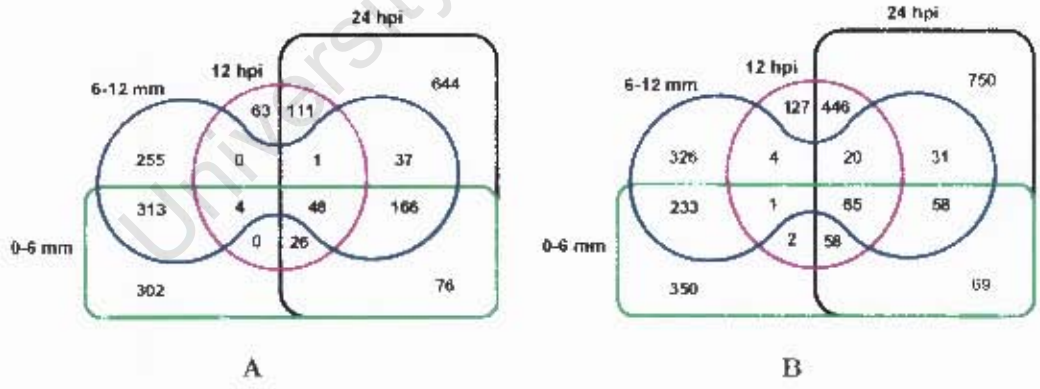


Figure 3.10: Genes significantly differentially up-regulated (A) and down-regulated (B) (cut-off of 2 fold or log of 1) after infection of *Arabidopsis* leaves with *B. cinerea* in the temporal and spatial microarray experiments were grouped into various categories. Green and black rectangles represent genes up- or down-regulated in the 0-6 mm distance (spatial experiment) and after 24 hpi (temporal experiment) respectively while the blue dumb bell and purple circle represent genes up- or down-regulated in the 6-12 mm distance (spatial experiment) and after 12 hpi (temporal experiment) respectively.

In the spatial experiment, SAM identified 2,482 and 7,827 genes to be significantly differentially regulated close to the lesion (0-6 mm) and away from the lesion (6-12 mm) respectively. Of the 2,482 genes, 1,355 were up-regulated and 1,127 down-regulated while of the 7,827 genes, 3,295 were up-regulated and 4,532 down-regulated. Because this experiment was biologically replicated only three times, genes up- or down-regulated more than two fold in at least two replicates including average were selected. Based on this criteria, 935 (Appendix. B.1) and 824 genes (Appendix. B.2) were selected as up-regulated close to and away from the lesion respectively while 836 (Appendix. B.3) and 738 (Appendix. B.4) genes were selected as down-regulated in areas close to and away from the lesion respectively. Like in the temporal experiment, genes up- and down-regulated at the two distances were divided into three categories, category 1 consisted of genes up- and down-regulated only close to the lesion, category 2 consisted of genes up- and down-regulated at the two distances (overlapping) while category 3 consisted of genes up- and down-regulated only away from the lesion. Of the up-regulated genes, category 1, 2, and 3 consisted of 404, 531 and 293 respectively (Fig. 3.10A) while of the down-regulated genes, category 1, 2 and 3 consisted of 479, 357 and 381 respectively (Fig. 3.10B).

Examples of up-regulated genes in category 1 included ABC transporters, ACC oxidase, ACC synthase, Bax inhibitor-1, AP2 transcription factors, disease resistance proteins, glycosyl hydrolase, legume lectins, *LOX1*, GSTs, *NIT4*, proteinase inhibitors, protein kinases, terpene synthase (*At4g20200*), tryptophan synthase and zinc finger proteins. Category 2 consisted of genes such as ACC oxidase, ABC transporters, *AOC*, *AOS*, anthranilate synthase, cellulose synthases, disease resistance proteins, *ERD12*, *ERF1*, *ERF4*, GSTs, glycosyl hydrolases, *PR4*, LEA proteins, *LOX*, *NIT2*, *NIT3*, *PDF1.1*, *PDF1.2a*, *PDF1.2b*, *PDF1.2c*, *PDF1.3* and *PDF1.5*, pectin methylesterase inhibitors, peroxidase, protein kinases, *SAG12*, terpene synthase (*At1g61120*), AP2, MYB, and WRKY transcription factors and zinc finger proteins while category 3 included basic endochitinase, disease resistance proteins, *ERD6*, *EDS5*, F-box proteins, GSTs, glycoside hydrolase, pectin methylesterase inhibitor, peroxidases, protein kinases, *PGIP2*, AP2, MYB and WRKY transcription factors, UDP-glucosyl transferases and zinc finger protein.

Up- and down-regulated genes in the temporal and spatial experiments were also compared to determine the extent of overlap. The essence of this comparison was to determine if some of the *Arabidopsis* genes are induced as well as repressed by *B. cinerea* infection both in time and distance. This analysis demonstrated a limited overlap especially between 12 hpi and 6-12 mm distance in the temporal and spatial experiments respectively (Fig. 3.10). For instance, considering genes specifically up-regulated close to and away from the lesion, 12 and 24 hpi; no genes overlapped between 0-6 mm and 12 hpi and 6-12 mm and 12 hpi while only 76 and 37 genes overlapped between 0-6 mm and 24 hpi and 6-12 mm and 24 hpi respectively. Similarly, of the genes down-regulated specifically close to and away from the lesion, 12 and 24 hpi; only 2 and 4 genes overlapped between 0-6 mm and 12 hpi and 6-12 mm and 12 hpi respectively while 69 and 31 genes overlapped between 0-6 mm and 24 hpi and 6-12 mm and 24 hpi respectively.

This prompted a comparison between all the genes up- and down-regulated between the spatial and temporal microarray experiments. A similar trend like the one observed in the first analysis was observed. Considering a distance of 0-6 mm, only 78 and 316 up-regulated genes overlapped between 0-6 mm and 12 and 24 hrs respectively of which 74 genes were common in the three experiments (0-6 mm, 12 and 24 hpi) (Fig. 3.11A) while 126 and 250 down-regulated genes overlapped between 0-6 mm and 12 and 24 hpi respectively of which 123 genes were common in the three experiments (Fig. 3.11B). Considering a distance of 6-12 mm, only 53 and 252 up-regulated genes overlapped between 6-12 mm and 12 and 24 hrs respectively of which 49 genes were common in the three experiments (6-12 mm, 12 and 24 hpi) (Fig. 3.11C) while 90 and 174 down-regulated genes overlapped between 6-12 mm and 12 and 24 hpi respectively of which 85 genes were common in the three experiments (Fig. 3.11D).

This limited overlap may be attributed to two reasons; first, the spatial experiment was conducted in three biological replicates and only genes significantly up-regulated by more than 2-fold in at least two replicates and average were considered. This stringent selection criteria could have eliminated some of the genes present in the temporal experiment. Secondly, the spatial experiment was conducted to probe if some of the *Arabidopsis* genes are expressed in restricted areas of the leaf

such that they could have been missed in the temporal experiment where the whole leaf was considered. This supposition is demonstrated by the expression profile of *At4g01370* encoding a MAP kinase involved in mediating responses to pathogen infection. There was no significant change in expression levels of this gene in the temporal experiment yet it was significantly up-regulated close to the lesion in the spatial experiment. Similar expression profiles could have occurred for other genes. These two experiments demonstrated that infection of *Arabidopis* with *B. cinerea* results in early induction as well as restricted expression of some genes. In general, it does not seem that close to the lesion represents an earlier time point and far away a later time point.

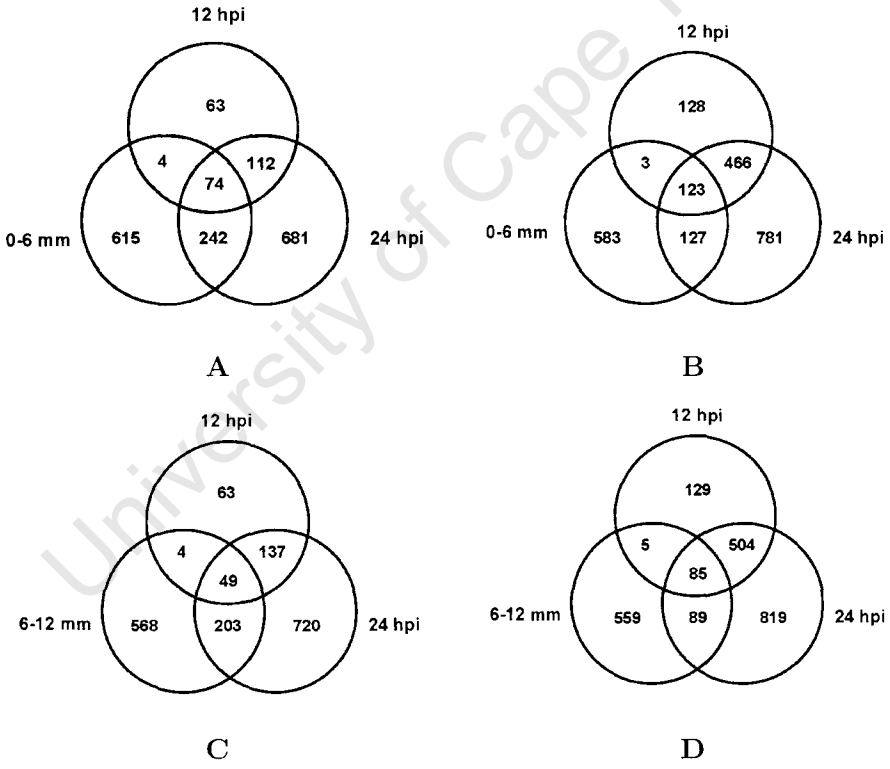


Figure 3.11: Comparison of genes up- (A and C) and down-regulated (B and D) in the spatial and temporal microarray experiments.

3.2.3 Validation of microarray results

3.2.3.1 Validation using quantitative PCR

The main purpose of validating microarray results is to associate the gene expression profiles observed in the microarray experiment to the biological differences between the samples under examination and not the technology. A number of technologies can be used to validate gene expression profiles however, quantitative PCR offers more advantages such as sensitivity, reproducibility, has a wide dynamic range and does not require any post-amplification manipulation (Abruzzo *et al.*, 2005; Wong and Medrano, 2005). Quantitative PCR was carried out on a subset of 24 genes of which 5 were down-regulated and 19 up-regulated in the temporal microarray experiment. All up-regulated genes were selected because they have been shown to have a role in host resistance to pathogens while the down-regulated genes encode proteins involved in photosynthesis and have been shown to be down-regulated in most host-pathogen interactions. Gene-specific primers for the selected genes were designed to amplify small fragments (70-150 bp) which result in high PCR efficiencies. Although the RNA extraction protocol that was used produces near pure RNA, primers were also designed to span an intron so as to check for any genomic DNA contamination (McPherson and Moller, 2006). Elimination of genomic DNA is very important because it eliminates overestimation of the amount of existing RNA. The same RNA samples used in the microarray experiments were used for quantitative PCR. Because of the cost factor, 12 hrs mock treated cDNA was used as a general reference hence 24 hrs mock was eliminated.

Like microarray, quantitative PCR is also affected by technical variations, which result from a number of factors that arise during reverse transcription and PCR reactions. The efficiency of reverse transcription is grossly affected by RNA quantity and quality while minor variations in reaction components, thermal cycling conditions, and mispriming events during the early stages of the reaction can lead to large changes in the overall amount of amplified product during a PCR reaction (Brunner *et al.*, 2004; Bustin and Nolan, 2004). Because of this, it is paramount that data resulting from quantitative PCR experiments is normalized. Normalization can be performed in a number of ways which include normalization to total

RNA, genomic DNA, ribosomal RNA especially 28S, however normalization to an internal reference or housekeeping gene is the most commonly used form of relative quantification (Wong and Medrano, 2005).

A number of studies have shown that the expression of housekeeping genes may vary with experimental conditions (Foss *et al.*, 1998; Schmittgen and Zakrajsek, 2000). A notable example is glyceraldehyde-3-phosphate dehydrogenase (GAPDH) one of the most common housekeeping genes that was significantly down-regulated in this study (Appendix A.2 and A.4). Consequently, normalization to a single housekeeping genes can falsely bias results; however, this problem can be minimized by using multiple housekeeping genes (Hellemans *et al.*, 2007). With multiple housekeeping genes, normalization is performed with a normalization factor that is calculated from the geometric mean of their expression levels (Hellemans *et al.*, 2007). Therefore, in addition to the 24 selected genes, four housekeeping genes; *At5g44200* (Nuclear cap-binding protein, *CBP20*), *At5g25760* (Ubiquitin ligase, *UBC21*), *At5g06600* (Ubiquitin-specific protease, *UBP12*), *At1g04820* (Tubulin, *TUA4*) were selected. Their selection was based on microarray normalized M values which showed that these genes were expressed at constant levels across the two time points (Table. 3.1).

Table 3.1: Log ratios for selected housekeeping genes obtained in the temporal microarray experiment. The experiment was replicated five times (Rep 1-5).

Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
12 hpi							
At1g04820	0.28	0.40	-0.15	-0.33	0.35	0.11	TUA4
At5g06600	0.48	0.02	-0.30	0.09	0.29	0.12	UBP12
At5g25760	0.09	-0.03	-0.22	0.07	0.09	-0.06	UBC21
At5g44200	0.05	-0.02	0.67	0.66	0.00	0.25	CBP20
24 hpi							
At1g04820	-0.43	0.20	0.56	-0.15	0.01	0.04	TUA4
At5g06600	0.35	0.11	0.32	0.16	0.12	0.21	UBP12
At5g25760	0.12	-0.10	0.27	0.02	0.09	0.08	UBC21
At5g44200	0.01	0.83	-0.09	-0.05	-0.16	0.11	CBP20

The first step was to confirm the stability of the assayed housekeeping genes. This was performed with BestKeeper, a Microsoft Excel applet (Pfaffl *et al.*, 2004). BestKeeper determines the optimal housekeeping genes through employing pair-wise correlation analysis of all pairs of candidate genes and calculates the geometric mean of the best-suited ones. It is freely available at www.genequantification.info. It uses raw Ct values generated by the quantitative PCR platform as estimators of expression levels because they are in most cases normally distributed, hence parametric tests can be performed on them (Pfaffl *et al.*, 2004). Descriptive statistics such as the geometric mean (GM), arithmetic mean (AM), minimum and maximum values, standard deviation (SD), and coefficient of variation (%CV) are computed. Also computed are the Min (x-fold) and Max (x-fold) values and their standard deviation. Min (x-fold) and Max (x-fold) values represent the number of times a particular gene has been over or under expressed from the geometric mean. A BestKeeper index (BKI) is also calculated; it is the geometric mean of all housekeeping gene Ct values. Ideally, consistent and stable housekeeping genes should have standard deviation values less than 1.

The candidate with the lowest variation in expression was *At5g25760* (CV: 0.74: SD: 0.15) and the highest variation was *At1g04820* (CV: 4.57; SD: 0.74). All these four candidates can be considered stable and therefore appropriate for normalization since their SD values were well below 1 (Table. 3.2). Because *At1g04820* showed a high up/downregulation of 1.67 fold in addition to high CV and SD values, it was hypothesized that it could be increasing the BKI1 CV (1.33%) and SD (0.24) values hence it may not make a good set of housekeeping genes. To prove or reject this hypothesis, housekeeping genes were removed one by one and their BKI examined. In all sets where *At1g04820* was retained, the CV and SD values increased to levels higher than when all the candidates were included (Table. 3.2). This indicated that the other three candidates were indeed compensating for variation in expression of *At1g04820*. The set involving *At5g06600*, *At5g25760* and *At5g44200* (BKI4) which had the lowest CV (0.79%) and SD (0.15) values was therefore selected.

Another open source software qBase (Hellemans *et al.*, 2007) was used for the calculation of the relative expression of the assayed genes. Data from the Rotorgene run for each gene was organized in a qBase standard format and exported

to qBase. Amplification efficiencies for each gene were calculated by means of linear regression and associated with the respective genes in a gene list. Because multiple housekeeping genes were used for normalization, their stability was also verified in qBase based on two parameters; the coefficient of variation and the gene stability measure (M) which is based on geNorm software (http://medgen.ugent.be/genorm).

Stable housekeeping genes from homogeneous samples are expected to have coefficient of variation and gene stability measure values less than 25% and 0.5 while those from heterogeneous samples, these values may increase to 50% for CV and 1 for M (Hellemans *et al.*, 2007). The three selected housekeeping genes recorded an average coefficient of variation of 21.73% and a gene stability measure value of 0.56 which confirmed this as a good set for normalization of target gene quantitative data (Table. 3.3). CV and M values obtained for *At5g06600* in the 1^{st} and 3^{rd} replicates were higher than those observed in the 2^{nd} replicate as well as those observed for *At5g25760* and *At5g44200* in all replicates. However these values were still within acceptable limits for a stable housekeeping gene. Following housekeeping gene selection, qBase calculates the average and standard deviation of the Ct values for all technical replicates which are then converted into relative quantities based on the gene specific amplification efficiencies. The relative quantities are normalized with a sample normalization factor which is calculated by taking the geometric mean of the relative quantities of the selected housekeeping genes. Normalized quantities are then rescaled relative to a calibrator which is a sample that was not treated (12 hrs mock). Relative quantities for each gene were log transformed to the base 2.

An average of the log transformed relative quantities from the quantitative PCR experiment were plotted with an average of log ratios from the first 3 replicates in the time course microarray experiment for easy comparison. The first three replicates were chosen because RNA from these same replicates was also used for quantitative PCR. A similar pattern in up- and down-regulation as observed in the microarray experiment was also observed for all the assayed genes in the quantitative PCR experiment (Fig. 3.12) and (Fig. 3.13). This demonstrated that the expression profiles observed in the microarray experiment were indeed not by chance or due to the technology but to the biological difference between the samples (infected and uninfected) and the three housekeeping genes provided a good set for normalization.

Table 3.2: Descriptive statistics of four candidate housekeeping genes based on their cycle threshold (Ct) values

Factor	*At1g04820*	*At5g06600*	At5g25760	*At5g44200*	BKI1 (n=4)	BKI2 (n=3)	BKI3 (n=3)	BKI4 (n=3)	BKI5 (n=3)
N	9	9	9	9	9	9	9	9	9
GM (Ct)	16.09	17.78	20.56	20.45	18.62	18.05	18.02	19.55	18.91
AM (Ct)	16.11	17.79	20.56	20.45	18.62	18.05	18.02	19.55	18.92
Min (Ct)	15.30	16.50	20.30	20.14	18.26	17.53	17.60	19.21	18.49
Max (Ct)	17.76	18.50	20.92	21.10	19.24	18.72	18.71	19.77	19.43
SD (±Ct)	0.74	0.45	0.15	0.28	0.24	0.29	0.29	0.15	0.37
CV (%Ct)	4.57	2.52	0.74	1.36	1.33	1.59	1.63	0.79	1.95
Min (x-fold)	-1.73	-1.88	-1.19	-1.21	1.26	1.38	1.29	1.14	1.32
Max (x-fold)	3.18	1.43	1.29	1.50	1.47	1.52	1.52	1.52	1.86
SD (± x-fold)	1.67	1.36	1.11	1.21	1.17	1.20	1.20	1.10	1.28

Abbreviations: N: number of samples; GM (Ct): the geometric mean of Ct; AM (Ct): the arithmetic mean of Ct; Min (Ct) and Max (Ct): the extreme values of Ct; SD (Ct): the standard deviation of the Ct; CV (%Ct): the coefficient of variance expressed as a percentage on the Ct level; Min (x-fold) and Max (x-fold): the extreme values of expression levels expressed as an absolute x-fold over- or under-regulation coefficient; SD (x-fold); standard deviation of the absolute regulation coefficients. **BKI1**: *At1g04820*, *At5g06600*, *At5g25760* and *At5g44200*; **BKI2**: *At1g04820*, *At5g06600* and *At5g25760*; **BKI3**: *At1g04820*, *At5g06600* and *At5g44200*; **BKI4**: *At5g06600*, *At5g25760* and *At5g44200*; **BKI5**: *At1g04820*, *At5g25760* and *At5g44200*

Table 3.3: Housekeeping gene quality determination with qBase

Locus	Rep 1	Rep 2	Rep 3	Mean
CV(%)				
At5g06600	30.59	18.63	29.92	26.38
At5g25760	21.40	15.39	19.30	18.70
At5g44200	21.71	16.38	22.27	20.12
Mean	24.57	16.80	23.83	**21.73**
M(geNorm)				
At5g06600	0.72	0.46	0.67	0.62
At5g25760	0.58	0.43	0.57	0.53
At5g44200	0.58	0.44	0.59	0.54
Mean	0.62	0.44	0.61	**0.56**

Abbreviations: CV (%): coefficient of variance, is the variation of the normalized relative quantities of a reference gene across all samples. Lower CV values denote higher stability; M: the gene expression stability parameter. The lower the M value, the more stably expressed is the reference gene

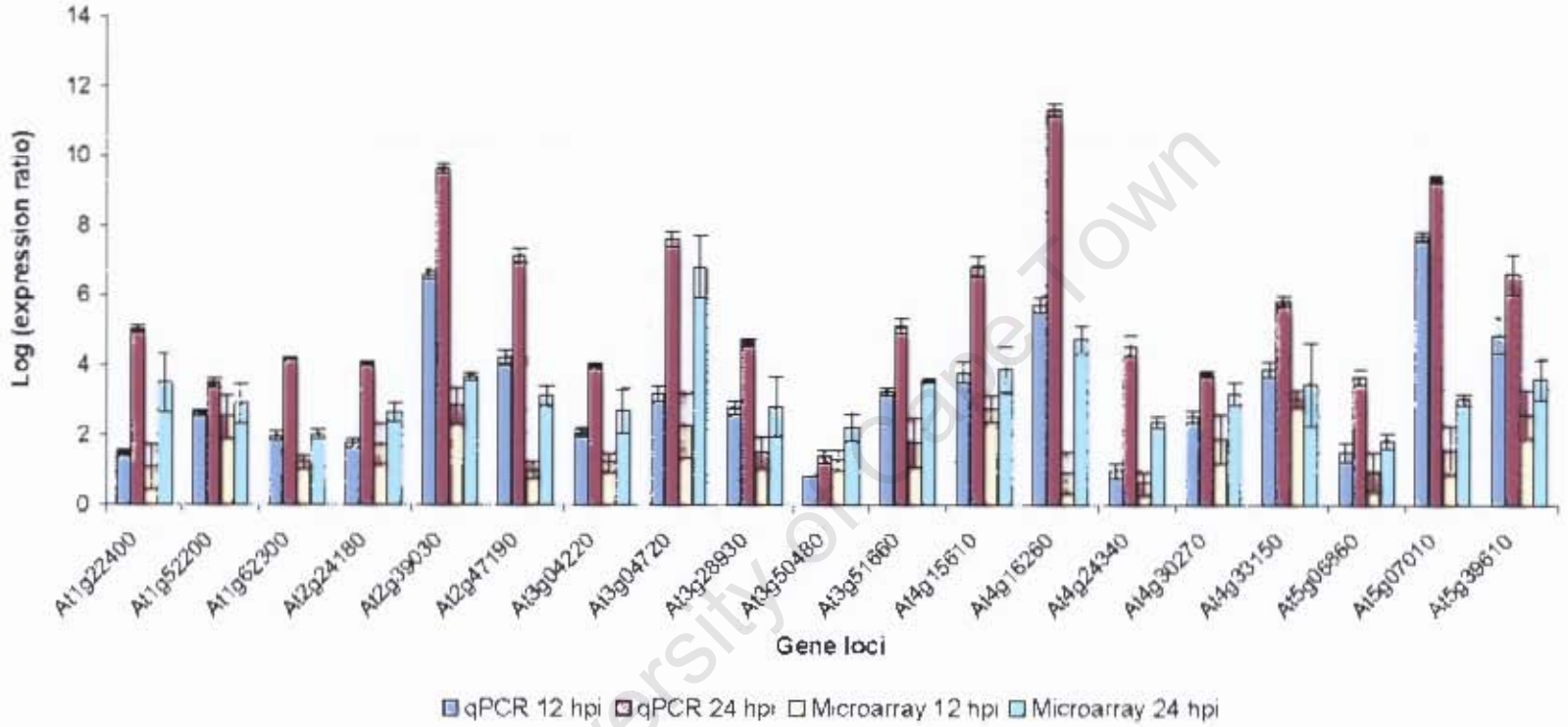


Figure 3.12: Expression levels of 19 up-regulated genes from the time course experiment after inoculation of *Arabidopsis* with *B. cinerea* as measured by quantitative PCR and Microarray. The y axis represents the log of the expression ratio: in microarrays, it is the log of the ratio of the red and green channels while in quantitative PCR, it is the log of the ratio of the normalized relative quantities of treated samples and normalized relative quantities of the calibrator (12 hrs mock). Therefore, each data point is an average of three replicates in the quantitative PCR and microarray experiments. Error bars represent standard errors.

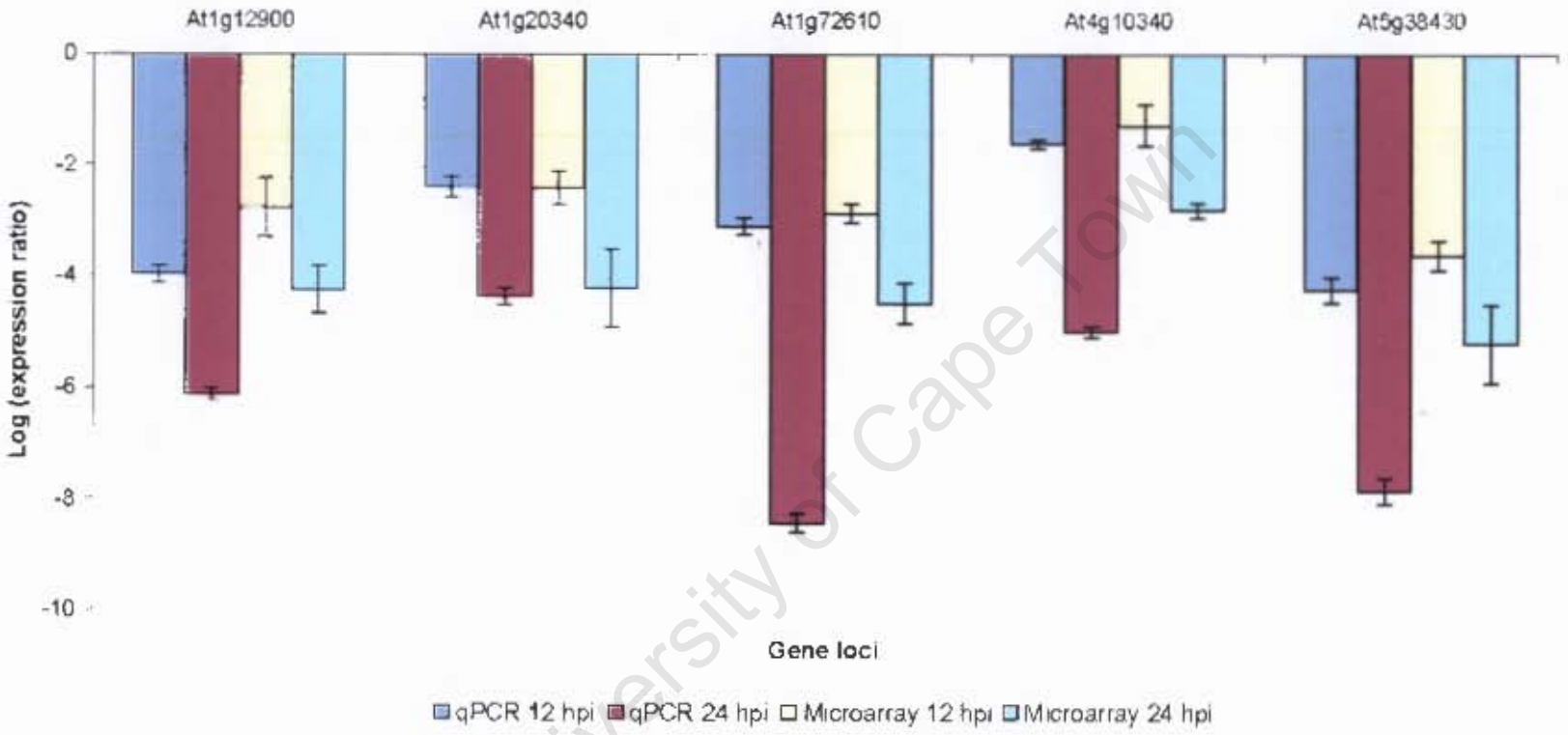


Figure 3.13: Expression levels of five down-regulated genes upon infection of *Arabidopsis* with *B. cinerea* as measured by quantitative PCR and Microarray. The y axis represents the log of the expression ratio; in microarrays, it is the log of the ratio of the red and green channels while in quantitative PCR, it is the log of the ratio of the normalized relative quantities of treated samples and normalized relative quantities of the calibrator (12 hrs mock). Therefore, each data point is an average of three replicates in the quantitative PCR and microarray experiments. Error bars represent standard errors.

3.2.3.2 Validation using *Arabidopsis* GUS lines

The expression pattern of additional genes was confirmed using promoter:reporter gene fusions where available. *Arabidopsis* plants containing the following constructs were obtained: *BGL2::GUS*, *CYP79B2::GUS*, *DOGT1::GUS* and *PAL::GUS*. *BGL2* (*PR2*) is induced following infection by biotrophic pathogens and is a marker for systemic acquired resistance (Glazebrook, 2001; Kunkel and Brooks, 2002). *CYP79B2* is involved in tryptophan metabolism which provides substrates for secondary metabolite biosynthesis, some of which have been shown to have antimicrobial activity. It catalyzes the conversion of tryptophan to indo-3-acetaldoxime (IAOx) (Fig. 3.18) (Glawischnig *et al.*, 2004). *DOGT1* encodes a UDP-glucosyl transferase which was shown to be involved in the detoxification of the *Fusarium* mycotoxin deoxynivalenol (Poppenberger *et al.*, 2003) while *PAL1* encodes an enzyme involved in the biosynthesis of SA necessary for local resistance to *B. cinerea* (Ferrari *et al.*, 2003).

Detached *Arabidopsis* infected and mock treated leaves were stained for GUS expression. Significant increases in expression was observed for *CYP79B2*, moderate increase in expression for *DOGT1* and *PAL1* and no increase in expression for *BGL2* after *B. cinerea* infection (Fig. 3.14). No GUS expression was detected around mock inoculated sites. This experiment was repeated with similar results. All these results show some consistence with what was observed in the two microarray experiments. *BGL2* (*At3g57260*) recorded only slight changes in expression in the temporal (log ratios of 0.14 (12 hpi) and 0.64 (24 hpi)) as well as the spatial experiment (log ratio of 0.03 (0-6 mm) and 0.26 (6-12 mm)). This behaviour in expression of *BGL2* was expected since this gene which is also referred to as *PR2* is known to be induced via the SA-signalling pathway which is more important in biotrophic infections than necrotrophic infections in *Arabidopsis*.

In the temporal experiment, *CYP79B2* (*At4g39950*) was significantly induced more than 4- and 8-fold after 12 and 24 hpi respectively while in the spatial experiment, it was induced by 2-fold close to the lesion but did not change in expression away from the lesion (log ratio of 0.18). This expression pattern is also demonstrated by GUS staining. *PAL1* (*At2g37040*) was induced 1.2- and 1.7-fold after 12 and 24 hpi respectively while in the spatial experiment, it was induced 2-fold

close to and away from the lesion however, based on the selection criteria it was not selected as significant. *DOGT1* (*At2g36800*) on the other hand was induced 10- and 18-fold after 12 and 24 hpi respectively, while in the spatial experiment, it was induced 3-fold close to the lesion and significantly down-regulated (log ratio of -1.12) away from the lesion. Although *DOGT1* was highly up-regulated in the temporal experiment, the moderate expression of GUS in the *DOGT1::GUS* transgenic line can be explained by its expression level observed in the spatial experiment.

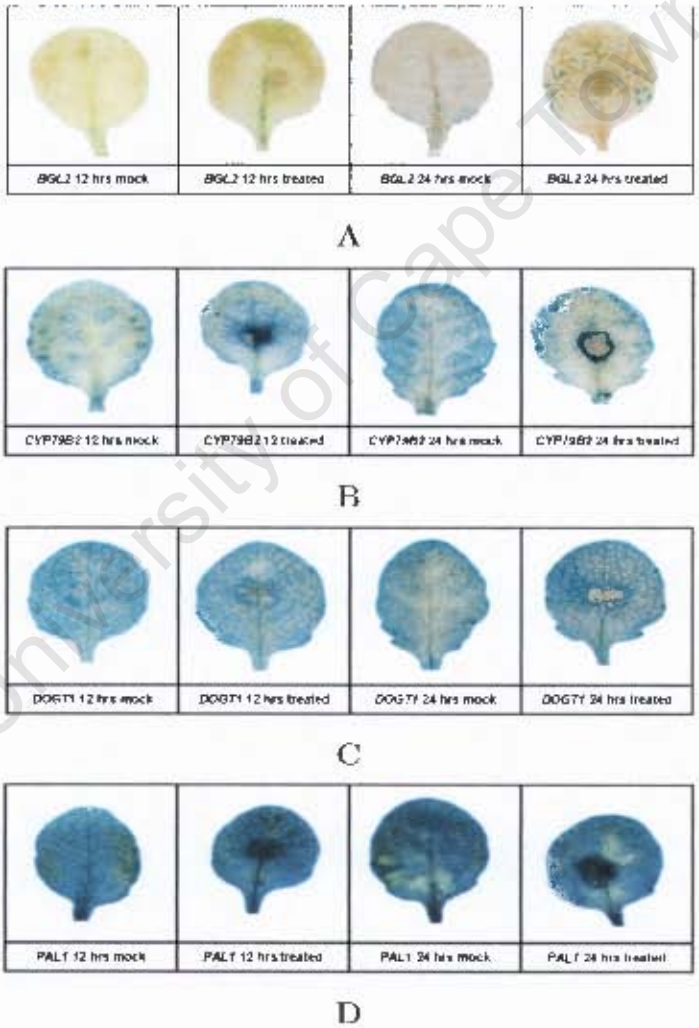


Figure 3.14: Four-week transgenic *Arabidopsis* leaves inoculated with *B. cinerea* and stained for GUS activity. The time points used in the temporal microarray experiment were also used in this experiment. **A** represents *BGL::GUS*; **B**, *CYP79B2::GUS*; **C**, *DOGT1::GUS* and **D**, *PAL::GUS* in the order of 12 hrs mock and treated and 24 hrs mock and treated.

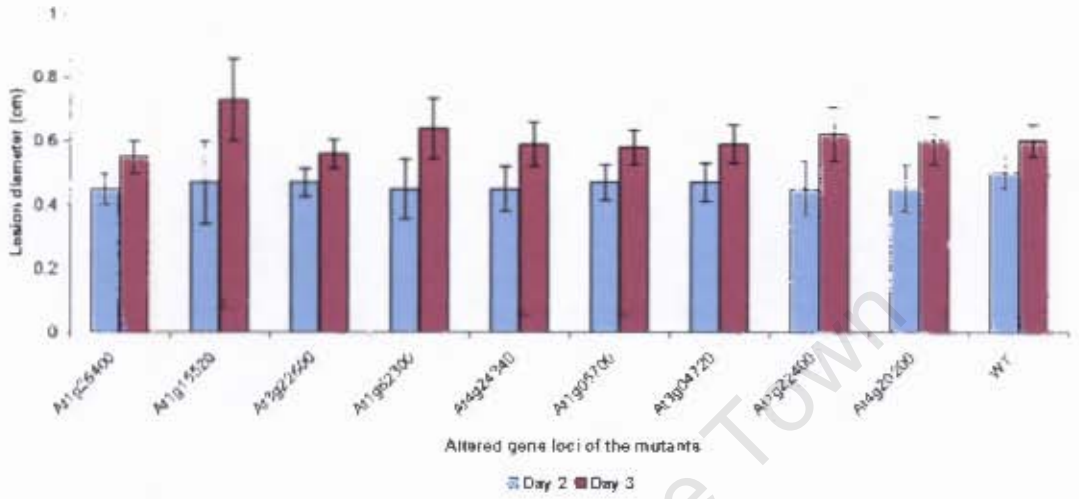
3.2.4 Role of identified genes in resistance

Deliberate creation of mutations within gene sequences and thereafter observing the effect of created mutations on the phenotype of the organism is one of the best ways of understanding gene function. This procedure, referred to as reverse genetics can be carried out in a number of ways which include targeted gene replacement, gene silencing and insertional mutagenesis (Koller *et al.*, 1989). Creating genome-wide gene disruptions with targeted gene replacement via homologous recombination has not been very successful in most higher eukaryotes (Parinov and Sundaresan, 2000; Gong and Rong, 2003). Gene silencing using RNA interference also presents several drawbacks which include lack of stable heritability of a phenotype and variable levels of gene activity (Wesley *et al.*, 2001; Hannon, 2002). Insertional mutagenesis using T-DNA and transposons has been used successfully to disrupt gene function in *Arabidopsis* (Sessions *et al.*, 2002; Alonso *et al.*, 2003; Grant *et al.*, 2003). It is based on the insertion of foreign DNA into the gene of interest often creating a null mutation (Krysan *et al.*, 1999; Radhamony *et al.*, 2005).

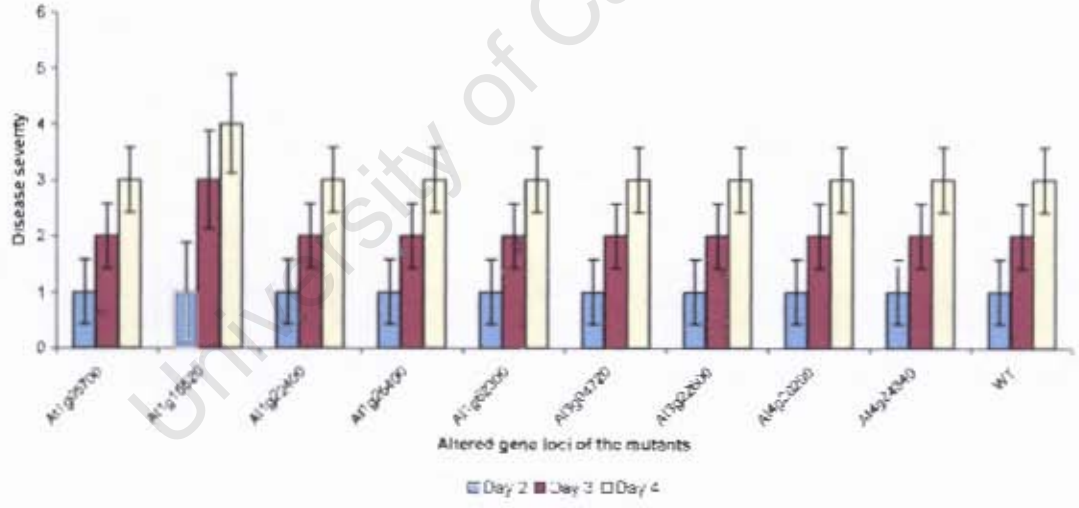
T-DNA insertion mutants for some of the significantly up-regulated genes in the temporal microarray experiment after 24 hrs were identified in T-DNA express (http://signal.salk.edu/cgibin/tdnaexpress). Twenty four T-DNA insertion lines were selected of which 3 were from the Syngenta Arabidopsis Insertion Collection (SAIL) (Sessions *et al.*, 2002) and 21 were from the Salk Institute (SALK) T-DNA collection (Alonso *et al.*, 2003). Selection of these mutants was based on position of T-DNA insertion within the sequence of the gene. Only mutants characterized by a T-DNA insertion in the 5'-UTR or the first half of the gene, which would more likely lead to a knockout mutation, were considered. Selected T-DNA insertional mutant lines were then obtained from the Nottingham Arabidopsis Stock Centre (http://arabidopsis.info). Lines homozygous for the insertion were confirmed through PCR. A PCR product ranging between 400-800 bp using genomic primers specific for the altered gene and a primer specific to the left border of the T-DNA indicated the presence of the T-DNA. The absence of a PCR product ranging between 900-1050 bp using primers specific to the altered gene designed across the T-DNA insertion, confirmed disruption of the gene and hence homozygosity of the insertion.

Nine lines were confirmed as homozygous for the insertion and were tested for altered susceptibility against *B. cinerea*. The wild-type line used for comparison was selected from the T-DNA insertion lines hence had the same background as the mutants. To determine the effect of the respective gene disruptions on the overall resistance phenotype of the plant, detached leaf (DL) and whole plant (WP) assays of four-week mutant *Arabidopsis* leaves and plants were conducted respectively. Based on the results, no difference in lesion diameters and disease severities on the second day in the two experiments and the third day in the WP assay experiment was observed however, N505635 altered in the gene *At1g15520* recorded slightly higher lesion diameters and disease severities compared to the wild-type on the third day in the DL assay and third and fourth day in the WP assay experiments. Lesion diameters and disease severities for the rest of the mutants on the second and third day in the DL and WP assay experiments respectively were comparable with those of the wild-type. This data is presented in Fig. 3.15.

The gene *At1g15520* (*AtPDR12*) encodes an ATP-binding cassette (ABC) transporter family protein that is localized in the plasma membrane and expressed only in leaves. Its expression has been reported to be induced by many other fungal pathogens (Stein *et al.*, 2006). ABC transporters are involved in many biological processes such as conferment of resistance to drugs and transport of compounds such as secondary metabolites and toxic substances Martinoia *et al.* (2002); van den Brule *et al.* (2002); Pighin *et al.* (2004); Yazaki (2006). Recently, Lee *et al.* (2005) demonstrated the involvement of *AtPDR12* in detoxification of lead(II). They reported a reduction in growth rate of mutants on lead(II)-containing medium. In addition, these mutants also had higher lead contents than wild-type plants. Over-expression of the gene resulted in plants resistant to lead(II) and had lower lead contents than wild-type plants. Expression of this gene has also been shown to be induced by many other fungal pathogens (Stein *et al.*, 2006). These results suggest *AtPDR12* gene product may have a role most probably in excreting plant toxic metabolites or pumping pathogen toxins out of the plant cells. However, because of the presence of other ABC transporters possibly with similar functions, the absence of *At1g15520* may have a minimal effect hence the mild phenotype. As this is a single insertion line, it would be important to identify a second independent line or complement the line that displayed a phenotype to be certain of the observed phenotype.



A



B

Figure 3.15: Lesion diameters (**A**) and disease severities (**B**) of *Arabidopsis* mutant leaves and whole plants respectively altered in the indicated genes after inoculation with *B. cinerea*. Data points in **A** represent average lesion diameters taken from a minimum of three leaves per mutant plant after the 2^{nd} and 3^{rd} day while in **B**, they represent average disease severity values taken from a minimum of three mutant plants after the 2^{nd}, 3^{rd} and 4^{th} day. Error bars in **A and B** represent standard error of the mean.

3.2.5 Functional categorization and MapMan visualization

To have a first look at the function of genes differentially up- and down-regulated significantly in the two experiments, functional categorization based on the biological process gene ontology was performed (Ashburner *et al.*, 2000). In this ontology, genes are organized based on the biological objective of their gene products (Ashburner *et al.*, 2000). Functional categorization was performed in FatiGO, a web-based application (http://fatigo.bioinfo.cipf.es) (Al-Shahrour *et al.*, 2004). Two lists of genes were uploaded into FatiGO to extract relevant gene ontology terms. The first list consisted of genes either up- or down-regulated whose ontologies were being investigated while the second list consisted of all *Arabidopsis* genes but without genes in the first list. The second list was used as a reference. Over-represented (proportion (%)) functional categories whose P values (cut-off of $P \leq 0.01$) had been adjusted for multiple testing were considered significant. The gene ontology level 3 was chosen since it constitutes a good compromise between information quality and number of genes annotated (Al-Shahrour *et al.*, 2004). Average expression values of genes significantly up- and down-regulated in the two experiments were also uploaded into MapMan (Thimm *et al.*, 2004) to get an overall picture of what is happening at the process level. MapMan is a user-driven tool and displays large expression data sets onto diagrams of metabolic pathways and other processes.

The functional category of response to chemical stimulus was over-represented in up-regulated genes of the temporal and spatial experiments while secondary metabolic process, defense response, and response to stress and external stimulus were over-represented in genes up-regulated after 24 hpi in the temporal experiment and both distances in spatial experiment (Table 3.4). The lack of representation of these functional categories in genes up-regulated after 12 hrs is possibly due to the low number of genes that displayed significant increases in abundance at this time point. The functional category of aging was over-represented in genes up-regulated after 24 hrs in the temporal experiment and close to the lesion in the spatial experiment. The most significant over-represented categories among down-regulated genes were photosynthesis, carbon utilization and biosynthetic process. The results demonstrate a strong overlap of gene expression in stress responses.

The over-representation of the functional category of secondary metabolic process is supported by the up-regulation of a number of genes encoding enzymes involved in the synthesis of secondary metabolites. For example, genes encoding enzymes involved in the biosynthesis of aromatic amino acids, the precursors of most of the secondary metabolites (Kitzing *et al.*, 2004) were up-regulated (Fig. 3.16 and 3.17). These aromatic amino acids are synthesized from chorismate, a product of the shikimate pathway (Herrmann and Weaver, 1999). A closer look at the shikimate pathway demonstrated that genes encoding enzymes involved in the synthesis of chorismate were significantly up-regulated (Fig. 3.18). It also demonstrated that tryptophan seems to be more important in the generation of secondary metabolites necessary for impeding *B. cinerea* as two of the genes encoding enzymes necessary for its synthesis from chorismate were significantly up-regulated (Zhao and Last, 1996; Zhao *et al.*, 1998) (Fig. 3.18).

Tryptophan is the precursor of indole-3-acetaldoxime (IAOx) from which camalexin, indole glucosinolates and indole-3-acetonitrile (IAN) are synthesized (Hull *et al.*, 2000; Bak *et al.*, 2001; Mikkelsen and Halkier, 2003; Glawischnig *et al.*, 2004). The gene encoding CYP71B15 which catalyzes the synthesis of camalexin was up-regulated after 12 hpi and significantly up-regulated after 24 hpi. The importance of camalexin is also demonstrated by the significant temporal up-regulation of At2g30770 shown to encode CYP71A13 necessary for catalyzing the conversion of IAOx to IAN in camalexin synthesis (Nafisi *et al.*, 2007). Indole glucosinolates are metabolized by myrosinases into biologically active nitriles, isothiocyanates, or thiocyanates (Wittstock and Halkier, 2002) while IAN is converted to indole-3-acetamide (IAM) and indole-3-acetic acid (IAA) by nitrilases (Vorwerk *et al.*, 2001; Kutz *et al.*, 2002; Pollmann *et al.*, 2002). Four nitrilase encoding genes (*NIT1*, *NIT2*, *NIT3* and *NIT4*) were up-regulated (Fig. 3.18) an indication that IAA could have been produced in this interaction. Other up-regulated genes encoding enzymes involved in the synthesis of secondary metabolites were the terpene synthases (Fig. 3.16 and 3.17). Terpene synthases catalyze the conversion of allylic prenyl diphosphate intermediates to a range of terpenoids (Facchini *et al.*, 2004). Examples of these genes included *At1g61120*, *At2g24210*, *At3g25830*, *At4g15870* and *At4g20200*. MapMan visualization also demonstrated the down-regulation of the photosynthetic photosynthetic pathway which supports results from functional categorization (Table 3.4).

Table 3.4: Functional categorization of up- and down-regulated genes in the two experiments based on biological process. The number and percentage represent the number and proportion of up- or down-regulated genes involved in a specific biological function (responsive genes) and the number and proportion of all genes in the genome involved in a similar biological function (whole genome). Only categories over-represented are considered.

	Responsive genes		Whole genome		
Entity	Number	Percentage	Number	Percentage	P-value
Up-regulated genes					
12 hpi					
Response to chemical stimulus	31	22.0	913	8.2	4.88 E-04
Response to biotic stimulus	16	11.4	285	2.6	7.24 E-04
24 hpi					
Response to chemical stimulus	103	19.5	841	7.9	3.59 E-13
Response to stress	82	15.5	733	6.9	1.20 E-08
Response to biotic stimulus	41	7.8	260	2.4	1.34 E-07
Secondary metabolic process	35	6.6	249	2.3	1.39 E-05
Response to external stimulus	24	4.6	150	1.4	1.43 E-04
Aging	12	2.3	42	0.4	3.20 E-04
0-6 mm					
Response to biotic stimulus	42	8.6	259	2.4	2.63 E-08
Response to chemical stimulus	84	17.3	860	8.0	4.78 E-08
Response to external stimulus	29	6.0	145	1.4	1.27 E-07
Response to stress	74	15.2	741	6.9	2.90 E-07
Immune system process	20	4.1	92	0.9	9.37 E-06
Secondary metabolic process	33	6.8	251	2.3	2.28 E-05
Defense response	44	9.1	407	3.8	3.40 E-05
Aging	11	2.3	43	0.4	8.17 E-04
Cellular response to stimulus	6	1.2	14	0.1	5.80 E-03
6-12 mm					
Response to external stimulus	33	7.8	141	1.3	9.26 E-12
Response to biotic stimulus	43	10.1	258	2.4	2.02 E-11
Response to stress	75	17.7	740	6.9	9.60 E-11
Response to chemical stimulus	79	18.6	865	8.0	3.55 E-09
Defense response	45	10.6	406	3.8	4.85 E-07
Secondary metabolic process	32	7.6	252	2.3	6.20 E-06
Immune system process	18	4.3	94	0.9	1.97 E-05
Response to abiotic stimulus	48	11.3	617	5.7	8.16 E-04
Down-regulated genes					
12 hpi and 0-6 mm					
Photosynthesis	36	8.4	43	0.4	3.38 E-27
Carbon utilization	12	2.8	3	0.0	3.21 E-12
Biosynthetic process	104	24.3	1,478	13.7	2.38 E-06
24 hpi					
Photosynthesis	52	7.0	27	0.3	3.62 E-39
Biosynthetic process	176	23.6	1,406	13.4	2.63 E-10
Carbon utilization	12	1.6	3	0.0	9.79 E-10
Nitrogen compound metabolic process	40	5.4	295	2.8	9.59 E-03
6-12 mm					
Photosynthesis	23	6.4	56	0.5	3.97 E-13

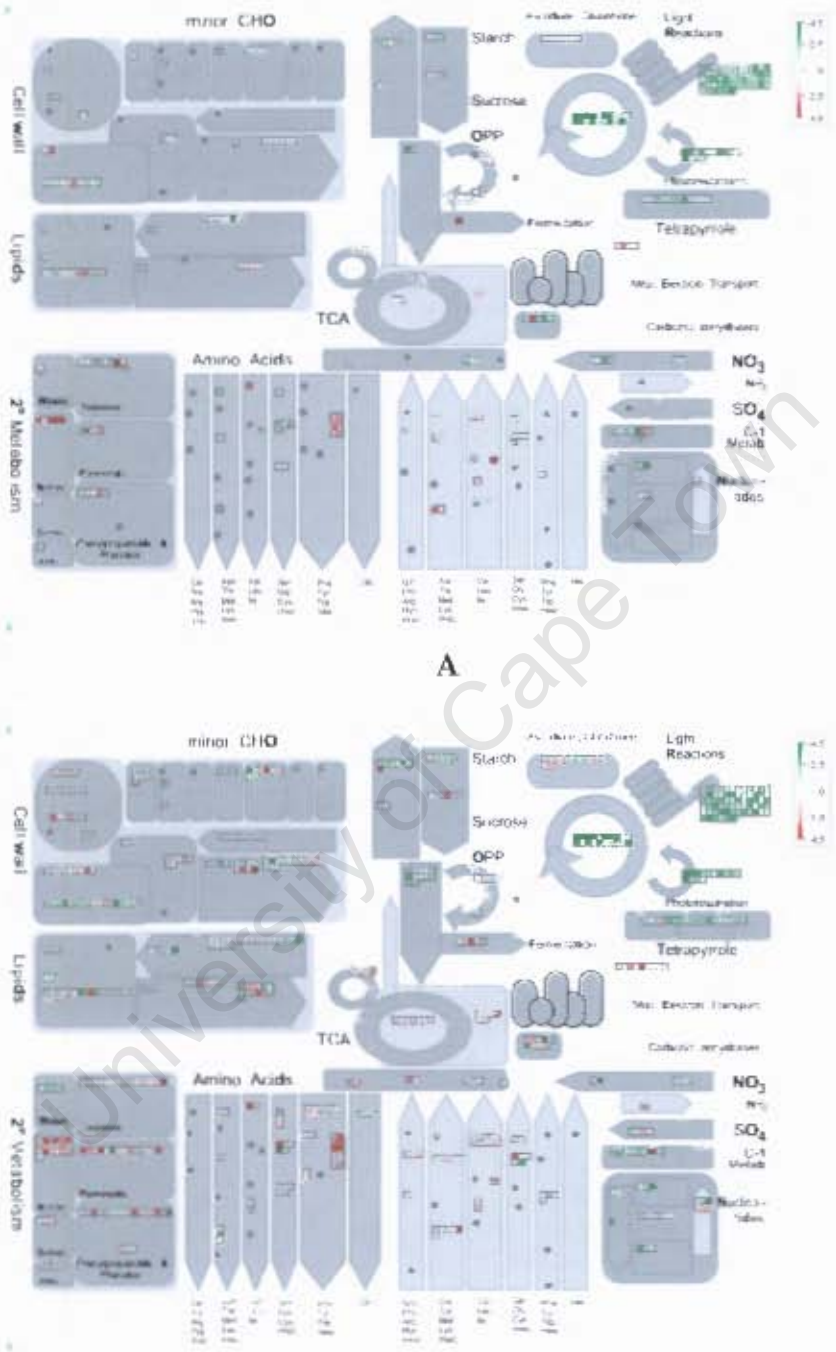


Figure 3.16: MapMan "Metabolism overview" display created using 1,003 (A) and 2,686 (B) significantly up- and down-regulated genes after 12 and 24 hrs respectively. Red and green squares represent up- and down-regulated genes respectively.

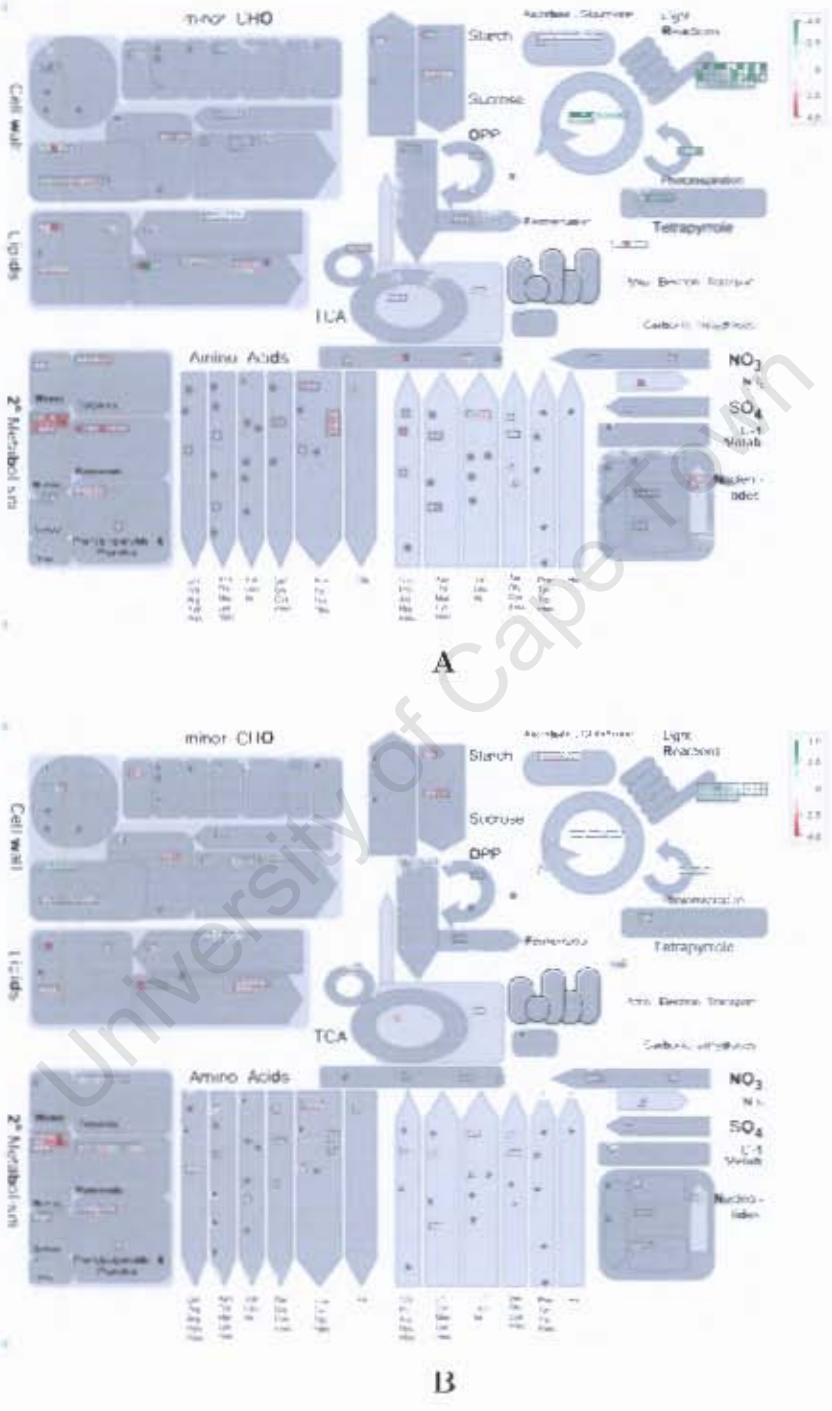


Figure 3.17: MapMan "Metabolism overview" display created using 883 genes (A) and 671 genes (B) which are only significantly up- and down- regulated close to the lesion (0-6 mm) and away from the lesion (6-12 mm) respectively

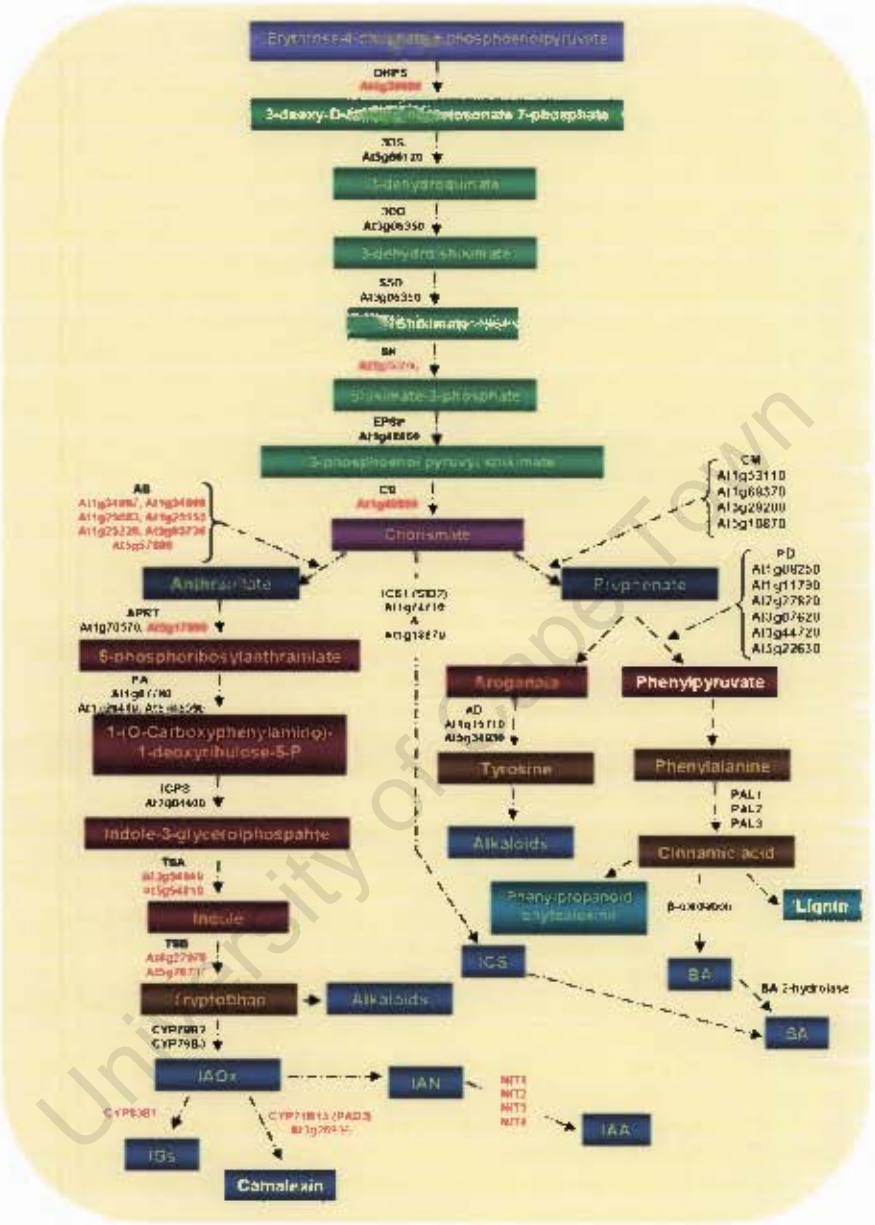


Figure 3.18: Differentially expressed genes modeled onto the shikimate biosynthetic pathway, the pathway through which secondary metabolites are generated. The pathway has been drawn based on AraCyc metabolic pathways available at TAIR (http://www.arabidopsis.org/biocyc/index.jsp). **Abbreviations**: **3DD**: 3-dehydroquinate dehydratase; **3DS**: 3-dehydroquinate synthase; **AD**: arogenate dehydrogenase; **APRT**: anthranilate phosphoribosyltransferase; **AS**: anthranilate synthase; **BA**: benzoic acid; **CM**: chorismate mutase; **CS**: chorismate synthase; **DHPS**: 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase; **EPSP**: 5-enolpyruvylshikimate-3-phosphate synthase; **IAA**: Indole-3-acetic acid; **IAN**: indole-3-acetonitrile; **IAOx**: indole-3-acetaldoxime; **ICS**: isochorismate synthase; **IGPS**: indole-3-glycerolphosphate synthase; **IGs**: indole glucosinolates; **NIT**: nitrilase; **PAI**: phosphoribosylanthranilate isomerase; **PAL**: phenylalanine ammonia lyase; **PD**: prephenate dehydrogenase; **S5D**: shikimate 5-dehydrogenase; **SA**: salicylic acid; **SK**: shikimate kinase; **TSA**: tryptophan synthase, alpha subunit; **TSB**: tryptophan synthase, beta subunit.

3.2.6 Promoter regulatory elements

Differences in expression patterns of genes largely depend on the number, order and type of protein binding sequences present in their promoters (Mahalingam and Fedoroff, 2003). These elements are referred to as motifs and motif analysis is often performed to identify these short conserved regulatory elements (Hulzink *et al.*, 2003; Wilson *et al.*, 2005). Identification of these elements is important especially in building gene regulatory networks controlling various processes such as defence. Since significant changes in transcript levels were observed, it was hypothesized that protein binding motifs associated with pathogen defence should be over-represented in the promoters of up-regulated genes compared with the total complement of *Arabidopsis* promoters. Therefore, 500-base pair sequences upstream of the ATG start codon were examined for over-represented 6-mer elements using the motif analysis program at TAIR (http://www.arabidopsis.org/tools/bulk/motiffinder/index.jsp).

The three categories of up-regulated genes in the temporal and spatial experiments (Section 3.2.2.4) were subjected to motif analysis. This analysis was carried out at TAIR (http://www.arabidopsis.org/tools/bulk/motiffinder/index.jsp). Category 1, 2 and 3 of the temporal experiment consisted of genes specific to 12 hpi (67), common to the two time points (186) and those specific to 24 hpi (923) respectively while category 1, 2 and 3 of the spatial experiment consisted of genes specific to 0-6 mm (404), common to the two distances (523) and those specific to 6-12 mm (290) respectively. A number of significant ($P \leq 0.01$) over-represented 6-mer elements were identified, however, only those that represent a known *cis* motif have been presented. Known *cis* motifs over-represented in promoters of genes up-regulated in the temporal experiment were the *Ocs* element, LS7, TGA1, G-box and W-box common in genes of category 2 and 3 while the MYB and ABA responsive element (ABRE) motifs were significant in category 2 and 3 respectively. All motifs from genes in category 1 were not significant possibly due to the small number of genes that constituted this category. Results are presented in Table 3.5. In the spatial experiment, elements over-represented but represent a known *cis* motif included the W-box and G-box (all categories); *Ocs* element and ABRE (category 1 and 2); MYC2 (category 2); TGA1 and LS7 (category 2 and 3) (Table 3.6).

Table 3.5: 6-mer elements over-represented in the 500 bp regions upstream of the ATG sites in the three categories of genes up-regulated in the temporal experiment that represent a known *cis* motif

		Absolute number of oligomer		Number of sequences containing oligomer		
Motif name	Oligomer	Query set	Genomic set	Query set	Genomic set	P-value
Category 1						
CACTTT	OBP-1	22	12941	15	10315	2.41E-02
ACGTGG	ABRE	20	5245	12	4214	6.88E-02
AAACCA	MYB1	47	21891	34	14986	7.86E-02
CACATG	MYC2	18	7574	16	6291	7.90E-02
TTGACT	W-box	30	13337	21	10320	1.03E-01
ACGTCA	LS7	11	4805	10	4060	1.17E-01
TAACTG	MYB2	11	5705	9	5101	1.20E-01
Category 2						
CACCAA	MYB	80	10156	70	8539	2.89E-04
ACGTAA	OCS	59	6633	50	5565	3.61E-04
ACGTCA	LS7	45	4805	37	4060	1.59E-03
TGACGT	TGA1	45	4805	37	4060	1.59E-03
CACGTG	G-box	66	7396	27	3097	9.13E-03
TACGTC	JASE2	25	2644	22	2451	1.23E-02
ACGTGG	ABRE	41	5245	32	4214	2.25E-02
TAACTC	MYB2	46	8155	34	6844	4.38E-02
CACTTT	OBP1	75	12941	62	10315	5.86E-02
TTGACT	W-box	84	13337	59	10320	6.21E-02
Category 3						
ACGTAA	OCS	260	6633	208	5565	1.05E-05
ACGTCA	LS7	186	4805	159	4060	1.29E-05
ACGTGG	ABRE	202	5245	164	4214	1.29E-05
TGACGT	TGA1	186	4805	159	4060	1.29E-05
CACGTG	G-box	300	7396	126	3097	2.03E-05
TTGACT	W-box	471	13337	344	10320	1.38E-04
CACTTT	OBP1	397	12941	316	10315	1.16E-02
AAACCA	MYB1	623	21891	424	14986	2.32E-02
TAACTC	MYB2	208	8155	188	6844	2.48E-02

Category 1 consists of up-regulated genes (67) specific at 12 hpi; category 2 consists of up-regulated genes (186) common to the two time points while category 3 consists of up-regulated genes (923) specific at 24 hpi.

Table 3.6: 6-mer elements over-represented in the 500 bp regions upstream of the ATG sites in the three categories of genes up-regulated in the spatial experiment that represent a known *cis* motif

		Absolute number of oligomer		Number of sequences containing oligomer		
Motif name	Oligomer	Query set	Genomic set	Query set	Genomic set	P-value
Category 1						
ACGTGG	ABRE	95	5245	78	4214	1.18E-04
ACGTAA	OCS	112	6633	92	5565	9.69E-04
TTGACT	W-box	210	13337	151	10320	3.41E-03
CACGTG	G-box	130	7396	53	3097	4.59E-03
CACATG	MYC2	75	7574	65	6291	1.04E-02
TAACTC	MYB2	113	8155	99	6844	1.40E-02
TGACGT	TGA1	75	4805	61	4060	1.94E-02
ACGTCA	LS7	75	4805	61	4060	1.94E-02
CACCAA	MYB	130	10156	115	8539	3.06E-02
Category 2						
TTGACT	W-box	305	13337	227	10320	8.80E-08
CACGTG	G-box	208	7396	88	3097	2.32E-07
ACGTGG	ABRE	134	5245	109	4214	6.64E-07
ACGTAA	OCS	153	6633	127	5565	2.81E-05
TGACGT	TGA1	101	4805	90	4060	8.20E-04
ACGTCA	LS7	101	4805	90	4060	8.20E-04
CACATG	MYC2	154	7574	126	6291	2.77E-03
TAACTC	MYB2	112	8155	101	6844	1.81E-02
CACCAA	MYB	176	10156	151	8539	2.50E-02
Category 3						
CACGTG	G-box	108	7396	48	3097	1.05E-04
TTGACT	W-box	154	13337	115	10320	1.91E-03
ACGTCA	LS7	61	4805	52	4060	2.78E-03
TGACGT	TGA1	61	4805	52	4060	2.78E-03
CACATG	MYC2	78	7574	67	6291	2.18E-02
TAACTC	MYB2	63	8155	54	6844	2.76E-02
CACTTT	OBP-1	111	12941	95	10315	4.96E-02
CACCAA	MYB	89	10156	78	8539	5.27E-02
ACGTGG	ABRE	54	5245	42	4214	5.57E-02

Category 1 consists of up-regulated genes (404) specific at 0-6 mm; category 2 consists of up-regulated genes (531) common to the two distances while category 3 consists of up-regulated genes (293) specific at 6-12 mm.

The over-representation of the W-box is supported by the up-regulation of genes encoding WRKY transcription factors. Eleven of the genes known to encode WRKY proteins (*WRKY6*, *WRKY18*, *WRKY33*, *WRKY40*, *WRKY45*, *WRKY48*, *WRKY53*, *WRKY58*, *WRKY60*, *WRKY61* and *WRKY75*) were significantly induced after *B. cinerea* infection. The W-box is common in promoters of many defence genes and WRKY transcription factors bind to it so as to mediate pathogen induced gene expression (Eulgem *et al.*, 1999; Maleck *et al.*, 2000). A number of WRKY proteins (e.g. WRKY18, WRKY22, WRKY29, WRKY33 and WRKY70) have been shown to have a direct role in plant defence against pathogens. For instance, WRKY22 and WRKY29 were shown to be involved in resistance responses to both bacterial and fungal pathogens (Asai *et al.*, 2002); constitutive expression of the *Arabidopsis WRKY18* (Chen and Chen, 2002) and *WRKY70* (Li *et al.*, 2004) resulted in enhanced expression of defense-related genes and increased resistance to virulent pathogens while mutation in and over-expression of WRKY33 resulted in enhanced susceptibility and resistance to *B. cinerea* and *A. brassicicola* respectively (Zheng *et al.*, 2006). It is therefore important that functional studies are carried out on genes encoding WRKY proteins that were significantly induced after *B. cinerea* infection to elaborate on their role in this interaction.

The *Ocs*-element is an enhancer element first identified in the promoter of the octopine synthase (*Ocs*) gene where it occurs as a 16 bp palindromic sequence (Bouchez *et al.*, 1989). This element is required for the expression of plant defense genes as well as pathogen genes in infected plants (Büttner and Singh, 1997). The *Ocs* element has been shown to regulate the transcription of plant GST genes which are now known to have a direct role in plant defence. Chen and Singh (1999) reported that this element mediates auxin, H_2O_2 and SA induced expression of the *GST6* promoter in *Arabidopsis*. Genes carrying the *Ocs* element included GSTs, peroxidases, proteinase inhibitors, transcription factors (WRKY and MYB), an indication that it is important in regulating transcription of these genes following infection. The *GST6* promoter was also shown to contain another motif OBP1 (OBF binding protein 1) which binds next to the OBF (*Ocs* element binding factors) binding site and is able to stimulate the binding of OBF proteins to the *GST6* promoter (Chen *et al.*, 1996). Although this motif was not significant, its presence in a multitude of genes up-regulated specifically after 24 hrs further suggests the importance of the

Ocs element in regulation of *Arabidopsis* genes induced by *B. cinerea*.

One of the most interesting observations was the over-representation of the ABA responsive element (ABRE) especially in genes up-regulated close to the lesion. This element is contained in promoters of many genes inducible by ABA and abiotic stresses such as dehydration, drought and wounding (Bonetta and McCourt, 1998; Leung and Giraudat, 1998; Shinozaki and Yamaguchi-Shinozaki, 2000). It serves as the binding site for the transcription factors AREBs (ABRE binding proteins) and ABFs (ABRE binding factors) (Yamaguchi-Shinozaki and Shinozaki, 2005, 2006). The cluster of genes in which the ABA responsive element was significantly over-represented in the spatial experiment was subjected to meta-analysis in genevestigator to determine the proportion of these genes likely to be induced by ABA. Close to 55% (60 out of 109 genes) of the genes in this cluster were also induced by ABA (data not shown). One of the genes in this cluster was *At2g33380* (*RD20*), this gene encodes a Ca^{2+} binding protein that has been shown to be induced by ABA treatment (Takahashi *et al.*, 2000). A similar analysis was carried out on all *Arabidopsis* genes significantly up-regulated by *B. cinerea* after 24 hrs. The results showed that 33.5% (348 out of 1,109 genes) of the *Arabidopsis* genes up-regulated after 24 hrs were also induced by ABA (Table 3.7).

The G-box and TGA1 motifs are binding sequences for bZIP transcription factors (Schindler *et al.*, 1992). The G-box motif resembles the ABA responsive element and functions in the regulation of plant genes in a variety of environmental conditions (Menkens *et al.*, 1995). It has also been shown to be important in regulation of genes that respond to jasmonate (Kim *et al.*, 1992; Mason *et al.*, 1993), a signalling compound that is now known to be important in mediating resistance responses to *B. cinerea* and other necrotrophs (Kunkel and Brooks, 2002; Glazebrook, 2005). The TGA subgroup of bZIP transcription factors has been shown to positively regulate INA-induced *PR1* expression. The LS7 motif is a TGA-bZIP factor binding site within the *PR1* promoter and was shown to be important for INA activation of *PR1*. It is positively induced by SA and is important for transcription activation of genes associated with systemic acquired resistance (Lebel *et al.*, 1998). The over-representation of these motifs TGA1 and LS7 suggests a role for the SA pathway in the *Arabidopsis*/*B. cinerea* interaction.

The motifs that were not significantly over-represented included JASE2 (JA/senescence-responsive element 2), MYB1, MYB2 and MYC2 in addition to OBP1 which has been discussed above. Although these motifs were not significantly over-represented, their presence provides an insight on the regulatory networks controlling resistance to *B. cinerea* in *Arabidopsis*. For instance, the motifs MYB1 and MYC2 are found in the promoter of the dehydration-responsive gene *rd22* (*At5g25610*). MYB1 is the MYB recognition site while MYC2 is the binding site for AtMYC2 (*rd22BP1*, *At1g32640*) in *rd22* (Yamamoto *et al.*, 2007). The role of AtMYC2 which encodes a nuclear-localized helix-loop-helix-leucine transcription factor has been well elucidated by Lorenzo and associates (2004) . They demonstrated that this transcription factor differentially regulates two branches in the JA signaling pathway one of which positively regulates expression of genes involved in response to wounding while the other negatively regulates expression of genes involved in pathogen defence. The MYB2 motif which was also not significantly over-represented is the binding site for ATMYB2, an *Arabidopsis* MYB homolog involved in regulation of genes that are responsive to water stress (Yamamoto *et al.*, 2007). JASE2 is found in the promoter of the *Arabidopsis* 12-oxo-phytodienoic acid-10,11-reductase (*OPR1*, *At1g76680*) gene. Together with JASE1, it is required for the up-regulation of *OPR1* by leaf senescence and JA (He and Gan, 2004). The role of JA in mediating host resistance to *B. cinerea* is known while the role of *B. cinerea* in induction of leaf senescence has also been elucidated (Swartzberg *et al.*, 2008). Put together, this analysis demonstrates that defence responses aimed at containing *B. cinerea* in *Arabidopsis* are very intricate involving a multitude of genes regulated by various elements.

3.2.7 Comparison of *Arabidopsis* gene expression profiles induced by *B. cinerea* with other biotic and abiotic stresses

The expression profiles of *Arabidopsis* genes induced by *B. cinerea* were also compared with those induced by other stresses. These stresses included biotic such as infection with pathogens and infestation with insect pests and abiotic such as treatment with phytohormones (ABA and IAA) and subjection to oxidative stress. For

biotic stresses, only experiments in which *Arabidopsis* leaf tissue was affected were considered for easy comparison. The criteria for selecting pathogens to include in the analysis was based on the pathogen mode of nutrition such as biotrophs (*E. orontii* and *Pseudomonas syringae* pv *tomato*), hemibiotroph (*P. infestans*) and a necrotroph (*A. brassicicola*). Insects considered in this analysis were the western flower thrips (*Frankliniella occidentalis*) and green peach aphids (*Myzus persicae*) (De Vos *et al.*, 2005). Arrays in which insect attack was investigated were included because JA and ET mediate defence against these pests like in *B. cinerea*. The rationale of including arrays in which *Arabidopsis* was subjected to oxidative stress and also treated with ABA and IAA was based on the fact that the oxidative burst plays a significant role in host colonization by *B. cinerea* while ABA and IAA seem to be produced in *Arabidopsis* following infection by *B. cinerea*.

Because the 24 hr time point showed the greatest number of statistically significant gene expression changes, it was considered for all downloaded experiments except for the *M. persicae* experiment where the earliest time point was 48 hrs and ABA and IAA experiments with a later time point after treatment of 3 hrs. Microarray data sets corresponding to all listed experiments were downloaded from the gene expression omnibus (http://www.ncbi.nlm.nih.gov/geo) and AtGenExpress (http://www.arabidopsis.org/info/expression/ATGenExpress.jsp). All downloaded experiments were performed using the GeneChip *Arabidopsis* ATH1 genome array (Redman *et al.*, 2004). This array contains 22,500 probe sets which represent approximately 24,000 gene sequences of *Arabidopsis* (http://www.affymetrix.com). The compressed cell intensity files (CEL) were uploaded into Expresso, a component of GEPAS (Herrero *et al.*, 2003) for quantile normalization. Experiments involving *A. brassicicola*, *F. occidentalis* and *M. persicae* were replicated once, ABA, IAA, oxidative stress and *P. syringae* pv *tomato* were replicated twice while those of *E. orontii* and *P. infestans* were replicated 3 times.

A list of genes up- or down-regulated by more than 2 fold on average after 24 hrs following infection of *Arabidopsis* with *B. cinerea* was compiled. Average log ratios for genes up- or down-regulated in the selected experiments were extracted and combined with the *B. cinerea* infection data into one spreadsheet with rows representing genes and columns representing experiments. Microarray experiments may

not be directly comparable because of a number of reasons which include differences in age of plants and type of tissue used, time points, micro-conditions and differences in microarray platforms. For example in this work, spotted oligonucleotide microarrays were used while in all downloaded experiments, Affymetrix microarrays were used. To make this data as comparable as possible, a z-score transformation was performed for each gene. In this transformation, the mean and standard deviation values were calculated for each row. This was followed by transformation of each value by subtracting the mean and dividing by the standard deviation.

In total 2,726 genes (rows) and 10 experiments (columns) were clustered based on experiments as well as experiments and genes. The essence of clustering based on experiments was to determine correlations between expression profiles induced by different stresses while clustering based on experiments and genes was to identify groups of co-regulated genes. Clustering based on experiments was carried out using hierarchical clustering (Eisen *et al.*, 1998). This was performed on elements within each experiment using uncentered Pearson's correlation and complete linkage clustering. The uncentered Pearson's correlation is the same as the Pearson correlation coefficient except that the sample means are set to equal to zero. It is the most appropriate distance metric where there is a zero reference state as in gene expression analyses where log-ratios are used and zero denotes no change in expression. Clustering based on experiments and genes was carried out using self organizing tree algorithm (SOTA). In this analysis, genes displaying similar trends in expression across treatments are grouped into clusters followed by hierarchical clustering of each cluster.

In addition to clustering, the meta-analysis program of the genevestigator database (Zimmermann *et al.*, 2004) was also used to analyze the response of the 1,109 *Arabidopsis* genes significantly differentially up-regulated after 24 hrs following infection by *B. cinerea*. The *Arabidopsis* arrays analyzed were the Affymetrix 22K arrays while the plant organs considered were rosette leaves at the 25-28.9 days. Stresses analyzed were abiotic (exposure to H_2O_2 and ABA) and biotic (infection with *Erysiphe cichoracearum*, *E. orontii* and *P. infestans* and infestation with *M. persicae*). Results from this analysis are presented in Table 3.7.

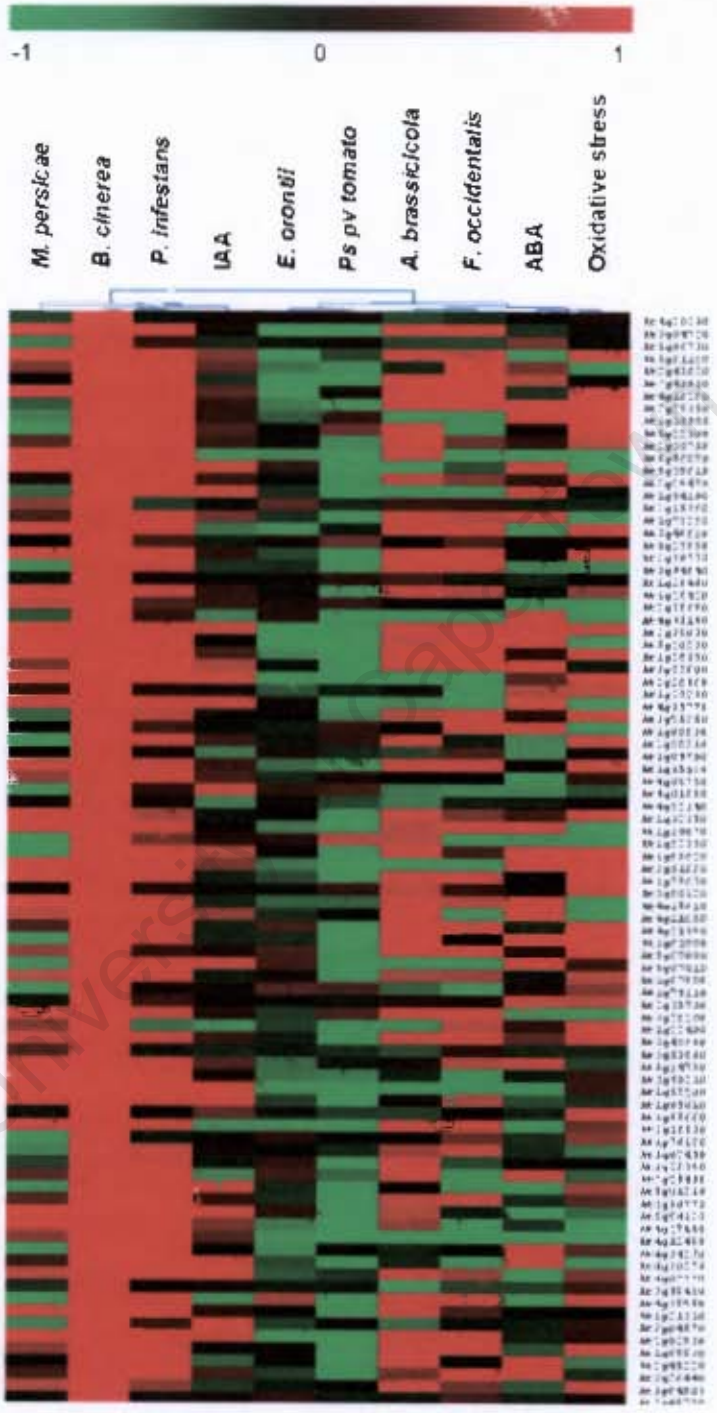


Figure 3.19: Hierarchical clustering of *Arabidopsis* gene expression profiles induced by different stresses. The whole image contains 2,726 genes hence only part of this image has been shown.

Clustering based on experiments demonstrated that expression profiles induced by *B. cinerea* were most similar to those induced by *P. infestans*, *M. persicae* and IAA. Similar expression profiles were observed between the insect pest *F. occidentalis* and the necrotroph *A. brassicicola*; the biotrophs *E. orontii* and *P. syringae* pv *tomato* as well as ABA and oxidative stress. Expression of genes in response to *A. brassicicola* was closer to the biotrophic pathogens than *B. cinerea*. Results from this analysis are presented in figure 3.19.

As indicated above, clustering based on experiments and genes was carried out to identify tightly co-regulated groups of genes with the view that such genes may be involved in accomplishment of a common function. Such co-regulated genes are important in breeding programs as they may target broad biotic as well as abiotic stresses. This analysis resulted in the grouping of genes into 11 clusters. Cluster 1 consisted of 572 genes the majority of which were up-regulated by *B. cinerea*, *P. infestans*, IAA, ABA, *M. persicae* and oxidative stress but down-regulated by *P. syringae* pv *tomato*, *A. brassicicola*, *E. orontii* and *F. occidentalis* (Fig. 3.20 and Appendix D.1). Examples included ACC oxidase and synthase, ABC transporter proteins, cellulose synthases, chitinases (these proteins targets chitin, a component of the fungal cell wall), disease resistance proteins, *ETR2*, *ERF1*, GSTs, glycosyl hydrolases, pectin methylesterase inhibitors, lectin protein kinases, MYB transcription factors (*MYB1*, *MYB7*, *MYB55*, *MYB108* and *MYB112*), *NIT4* and *OSM34*, peroxidases and zinc finger proteins (Appendix D.2). Functional categorization of genes in this cluster based on biological function demonstrated that the functional categories of macromolecule metabolic process and response to chemical stimulus were over-represented (Fig. 3.8) while motif analysis of genes in this cluster demonstrated that among the known motifs, the ABRE, G-box, LS7, MYB and TGA1 were the most over-represented (Fig. 3.9).

Cluster 2 consisted of 86 genes the majority of which were up-regulated by all fungal pathogens, ABA and oxidative stress but down-regulated by *P. syringae* pv *tomato*, *F. occidentalis* and *M. persicae* and IAA (Fig. 3.20 and Appendix D.1). Examples included chitinase (At3g47540), lectin protein kinase, *LOX1*, *NIT3*, pectin acetylesterase and transcription factors (bZIP (At1g75390 and At4g34590) and MYB (At1g32240)) (Appendix D.2). Functional categorization did not return any signif-

icant functional categories while motif analysis demonstrated that only the G-box was the most significantly over-represented motif (Fig. 3.9). Cluster 3 was made up 62 genes which were up-regulated by *B. cinerea*, *P. infestans*, ABA and oxidative stress but down-regulated by *A. brassicicola*, *P. syringae* pv *tomato*, *F. occidentalis* and *M. persicae* and IAA (Fig. 3.20 and Appendix D.1). Examples included a proteinase inhibitor/lipid transfer protein (LTP) (At2g48140), GSTs (At1g59700 and At2g29440), zinc finger protein (At5g01520) and protein phosphatases (At2g29380 and At5g59220) (Appendix D.2). Non of the functional categories obtained from functional categorization was significant while the ABRE, *Ocs* element, TGA1 and LS7 were the most significantly over-represented motifs (Fig. 3.9).

Cluster 4 consisted of 235 genes the majority of which were up-regulated by *A. brassicicola*, *E. orontii*, *P. syringae* pv *tomato*, ABA, and oxidative stress but down-regulated by *B. cinerea*, *P. infestans*, *M. persicae* and IAA (Fig. 3.20 and Appendix D.1). Examples included the dehydration-responsive protein (*rd22*, At5g25610), catalase 2 (At4g35090), expansin (At1g69530), starch synthase (At1g32900) (Appendix D.2). Functional categorization did not return any significant functional categories while motif analysis demonstrated that the most significantly over-represented motifs were ABRE, *Ocs* element, G-box and W-box. Cluster 5 was made up 192 genes which were up-regulated by all treatments except the biotrophs *P. syringae* pv *tomato* and *E. orontii* (Fig. 3.20 and Appendix D.1). Examples included ABC transporter (At1g15520), chitinases, GSTs, *PR1*, *PR4*, *PDF1.1*, *PGIP1*, protein kinases, terpene synthase (At1g61120), *WAK1*, WRKY transcription factors and Zinc finger proteins (Appendix D.2). No significant functional categories were observed in this cluster while the *Ocs* element and W-box were the most significantly over-represented motifs (Fig. 3.9).

Clusters 6, 7, 8 and 9 consisted of the least number of genes with cluster 6 consisting of 30 genes, cluster 7, 35 genes, cluster 8, 26 genes and cluster 9, 23 genes. Genes in these clusters were up-regulated by *B. cinerea*, *P. infestans* and *M. persicae* and IAA (Fig. 3.20 and Appendix D.1). A number of genes in cluster 7 were also up-regulated by *F. occidentalis* while those in cluster 9 were up-regulated by ABA. A number of genes in all these clusters seem to be down-regulated by *A. brassicicola*, *E. orontii*, *P. syringae* pv *tomato*, ox-

idative stress and *F. occidentalis* for clusters 6, 8 and 9 (Fig. 3.20 and Appendix D.1). Examples in cluster 6 included ACC oxidase (At1g05010), catalase 3 (*SEN2*, At1g20620), peroxidase 21 (At2g37130), proteinase inhibitors/LTPs (At4g12500, At4g12480, At4g12490 and At4g12470), mitogen-activated protein kinase (At4g08470) and ethylene response sensor (At2g40940); cluster 7, GST-related (At4g19880), ABC transporter (At1g59870), lectin protein kinase (At4g04960), protein kinase (At1g33770), GST (At2g02930), patatin (At2g26560), WRKY family transcription factor (At4g31800) and LEA3 (At4g02380); cluster 8, *SEN1* (At4g35770), pectinacetylesterase (At4g19420) and zinc finger proteins (At5g44260 and At4g11360) and cluster 9, glutathione peroxidase (At4g11600), bZIP family transcription factor (At5g49450), carbonic anhydrase (At1g58180). Zinc finger protein (At4g35480) (Appendix D.2). Functional categorization of all these clusters (6-9) did not return any significant functional categories possibly because of the low gene numbers while motif analysis demonstrated that only cluster 6 had a significantly over-represented motif (OBP1) while the rest (7-9) had non (Fig. 3.9).

Clusters 10 and 11 consisted of 691 and 774 genes respectively. The majority of genes in these clusters were down-regulated by all treatments except the biotrophs *E. orontii*, *P. syringae* pv *tomato* and oxidative stress (Fig. 3.20 and Appendix D.1). Examples in cluster 10 included 40S ribosomal proteins, 50S ribosomal proteins, 60S ribosomal proteins, auxin-responsive proteins, calcium-dependent protein kinases, ethylene receptor 1, glyceraldehyde-3-phosphate dehydrogenase B, starch synthases, nitrate reductase 1 and 2 while those in cluster 11 included auxin-responsive proteins, carbonic anhydrase, chlorophyll A-B binding protein, early-responsive to dehydration (ERD), expansin, F-box family proteins, *GER1*, *GER1*, *GAPA*, immunophilin, pectin methylesterase inhibitors, oxygen-evolving enhancer protein 1-1, ribosomal proteins, RuBisCO activase, starch synthase and thioredoxin (Appendix D.2). Functional categorization of cluster 10 and 11 demonstrated that the functional category of biosynthetic process was over-represented in both clusters while photosynthesis and carbon utilization were over-represented in cluster 11 (Fig. 3.8). Motif analysis on the other hand showed that the ABRE, G-box, MYC2 and W-box were significantly over-represented in genes of both clusters while OBP1 was over-represented only in genes of cluster 10 (Fig. 3.9).

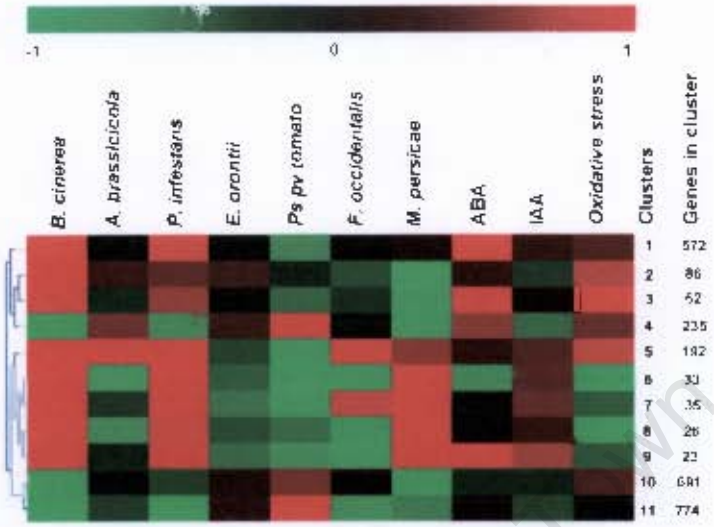


Figure 3.20: Cluster view of all clusters obtained by analyzing *Arabidopsis* genes induced and repressed by *B. cinerea* and other stresses. Cluster analysis was performed with self organizing tree algorithm (SOTA) and hierarchical clustering in TIGR multi experiment viewer using uncentered Pearson's correlation and complete linkage. Clusters are represented in rows while experiments in columns. The time points considered for all the experiments was 24 hr except for *M. persicae* whose earliest time point was 48 hrs while 3 hr, the last time point in ABA and IAA experiments was selected.

Table 3.7: Analysis of 1,109 *Arabidopsis* genes up-regulated by *B. cinerea* after 24 hpi to determine their response to other abiotic and biotic stresses using the meta-analysis program of genevestigator database

	Up-regulated genes		Down-regulated genes	
Stress	**Number**	**Percentage**	**Number**	**Percentage**
E. cichoraceurum	354	34.1	212	20.4
E. orontii	177	17.0	162	15.6
M. persicae	212	20.4	217	20.9
P. infestans	529	50.9	139	13.4
P. syringae	510	49.1	108	10.4
H_2O_2	393	37.8	196	18.9
ABA	348	33.5	224	21.6
ET	331	31.9	187	18.0
MeJA	333	32.1	190	18.3
SA	364	35.0	235	22.6

Motif name	Oligomer	Absolute number of oligomer		Number of sequences containing oligomer		P-value
		Query set	Genomic set	Query set	Genomic set	
Cluster 6						
CACTTT	OBP1	23	12941	17	10315	3.29E-03
Cluster 10						
CACGTG	G-box	212	7396	92	3097	3.49E-04
ACGTGG	ABRE	129	5245	107	4214	8.25E-03
CACATG	MYC2	139	7574	113	6291	3.77E-03
CACTTT	OBP1	311	12941	248	10315	3.38E-03
Cluster 11						
CACGTG	G-box	246	7396	106	3097	6.56E-05
ACGTGG	ABRE	190	5245	135	4214	1.21E-04
TTGACT	W-box	266	13337	213	10320	5.83E-04
CACATG	MYC2	207	7574	171	6291	8.19E-03

This analysis demonstrated that expression profiles of *A. brassicicola* were closer to the biotrophs than a similar necrotroph *B. cinerea*. The limited resemblance may be attributed to the fact that *Arabidopsis* is nonhost to this pathogen. On the other hand, expression profiles of the hemibiotroph *P. infestans* were similar to those of *B. cinerea* in all the clusters. These results are also supported by those from meta-analysis which showed that more than half (50.9%) of the genes significantly induced by *B. cinerea* were also induced by *P. infestans* (Table. 3.7). As indicated at the beginning of this subsection, *P. infestans* is a hemibiotrophic fungal pathogen, these kind of pathogens initiate their infections with an initial period of biotrophy followed by a necrotrophic phase in which hyphae ramify through host tissues killing them in advance of their spread (Perfect and Green, 2001). *Arabidopsis* is also host to this pathogen. These two put together (i.e. necrotrophic life style and *Arabidopsis* being a host) possibly explain the similarity in expression profiles of *B. cinerea* and *P. infestans*.

The biotrophs *E. orontii* and *P. syringae* pv *tomato* produced similar expression profiles however, they were different from those of *B. cinerea*. Modes of infection by biotrophic pathogens are different from those of necrotrophic pathogens, as a result, these two types of pathogens are bound to induce different defence responses. For instance, resistance to biotrophic pathogens in *Arabidopsis* is mediated by SA while ET and JA in addition to SA mediate resistance to necrotrophs

(Glazebrook, 2005). Meta-analysis showed that a slightly high proportion (49.1%) of *Arabidopsis* genes induced by *B. cinerea* were also induced by *P. syringae* (Table. 3.7). The reason for this discrepancy may be attributed to the fact that in genevestigator, data sets from all time points are merged which may lead to differences in the results.

The insect pest *M. persicae* displayed expression profiles similar to those of *B. cinerea* however, the other insect pest *F. occidentalis* demonstrated limited similarity. Meta-analysis also showed that 20% of the genes induced by *B. cinerea* were also up-regulated by *M. persicae* (Table. 3.7). This similarity may be attributed to the fact that resistance to insect pests is mediated by the JA/ET-dependent signalling pathway like many other necrotrophs including *B. cinerea*. For instance, feeding by *M. persicae* was shown to induce expression of JA as well as SA responsive genes (Moran and Thompson, 2001). The *Arabidopsis* mutant *cev1*, which constitutively expresses JA responsive genes, supports less *M. persicae* populations (Ellis *et al.*, 2002) while *coi1*, the *Arabidopsis* JA-insensitive mutant supports higher populations. The tomato mutant *def1* compromised in JA signalling, displays enhanced susceptibility while overexpression of the JA-inducible prosystemin resulted in enhanced resistance (Li *et al.*, 2002).

The phytohormone IAA seems to play a role in this interaction as ones encoding enzymes involved in its biosynthesis were up-regulated. Clustering based on experiments demonstrated that expression profiles induced by IAA were close to those induced by *B. cinerea* while clustering based on experiments and genes showed a similar pattern in expression profiles in 9 clusters. Another phytohormone that may play a role in this interaction is ABA. Its role is supported by the significant over-representation of the ABA responsive element (Fig. 3.6) and meta-analysis which showed that 33.5% of the genes significantly up-regulated by *B. cinerea* were up-regulated by ABA (Table. 3.7). The fact that these two phytohormones lead to induction of a multitude of genes which are also induced by *B. cinerea* suggests that they play significant roles in this interaction either to the benefit of the host or the pathogen. However, what can not be determined is whether they originate from the host as a result of primary or secondary effects of infection or from the pathogen.

Studies by Govrin and Levine (2000) demonstrated that the HR orchestrated by the oxidative burst plays a significant role in promoting host colonization in *B. cinerea*. Although clustering based on experiments showed that expression profiles induced by oxidative stress were distant from those induced by *B. cinerea*, clustering based on experiments and genes showed that *Arabidopsis* genes in 4 clusters were up-regulation by both *B. cinerea* and subjection to oxidative stress. The role of oxidative stress in *B. cinerea* interactions is further demonstrated by meta-analysis which showed that more than one third (37.8%) of the genes significantly induced by *B. cinerea* were also induced by H_2O_2 (Table. 3.7).

The majority of genes down-regulated by *B. cinerea* were clustered in clusters 10 and 11. Functional categorization of genes in cluster 11 showed the over-representation of the functional category of photosynthesis in addition to those of biosynthetic process and carbon utilization. An interesting observation is that the majority of genes contained in these 2 clusters were not down-regulated by the biotrophic pathogens. This suggests that certain pathways may be down-regulated in some interactions but up-regulated in others. This pattern of resistance although observed in *Arabidopsis* may be also be present in other plants. As indicated in subsection 3.2.6, the W-box and OBP1 are present in promoters of many genes with roles in defence. This suggests that like up-regulation, down-regulation of genes or pathways is a resistance response.

The overlap of gene expression in stress responses that was mentioned in subsection 3.2.5 can also be observed in this analysis as the ABA responsive element which is known to be present in promoters of genes induced by various abiotic stresses was also significantly over-represented in a number of clusters in which *Arabidopsis* gene were up- or down-regulated by biotic stresses. This point of overlap in stress responses is further strengthened by the over-representation of the G-box in a number of clusters. This motif is known to regulate plant genes in a variety of environmental conditions. Other motifs over-represented included LS7 and TGA1 and *Ocs* element. The over-representation of LS7 and TGA1 motifs especially in clusters in which *Arabidopsis* genes were up-regulated by *B. cinerea* further demonstrates the importance of SA in *Arabidopsis* resistance to *B. cinerea*.

One of the aims of gene expression profiling is to identify candidate genes for use in breeding programs. This analysis has shown that genes in clusters 1, 2, 3, 5-9 are candidates that may play a role in host resistance to *B. cinerea*, and *P. infestans* and *M. persicae* (clusters 5-9). Genes in cluster 2 may be important in host resistance to a number of fungal pathogens while those in cluster 5 to the necrotrophs, hemibiotrophs and insect pests. The analysis has demonstrated the relationship between *Arabidopsis* gene expression profiles induced by different stress.

3.3 Discussion

Two microarray experiments were set up to study the interaction of *B. cinerea* and *Arabidopsis* with the ultimate aim of identifying genes that may have a role in resistance. The first experiment focused on induction and repression of *Arabidopsis* genes after infection over a time course while the second experiment focused on spatial expression of *Arabidopsis* genes responsive to *B. cinerea*. By minimizing experimental variation and employing an experimental design involving not less than three biological replications in either experiment; it was possible to identify an array of genes that were induced during this interaction. Similarly a multitude of genes were down regulated. This down-regulation can be a positive step and results in the required gene expression changes such as repressors. The consistency and reliability of microarray results was confirmed for a subset of 28 genes from the temporal experiment using quantitative PCR. A similar pattern in expression to that of the microarrays was observed for all assayed genes. *Arabidopsis* plants carrying promoter:reporter gene fusions for some of the genes, were also infected with *B. cinerea*. The outcome from this experiment also conformed to the microarray results.

Functional categorization showed that secondary metabolic processes, response to chemical stimulus, defence, stress and external stimulus functional categories were the most over-represented among the up-regulated genes while photosynthesis, carbon utilization and biosynthetic process were over-represented among the down-regulated genes. Motif analysis of genes significantly up-regulated showed that the most significant over-represented known motifs were the ABA responsive element, the W-box, MYB and the OCS element. Analysis of expression profiles

of various abiotic and biotic stresses demonstrated that expression profiles of *B. cinerea* were closer to those of *P. infestans* than *A. brassicicola*. The insect pests especially *M. persicae* up-regulated a number of genes like *B. cinerea* an indication that they induce similar defence responses hence similar strategies may be deployed in containing them. The phytohormones ABA and IAA also up-regulated a number of genes up-regulated by *B. cinerea* an indication that these compounds are involved in host resistance to *B. cinerea* in which they possibly act as pathogenicity factors produced in the host as a primary or secondary response to pathogen invasion or through manipulation of host activities by the pathogen.

3.3.1 PGIPs in host resistance to *B. cinerea*

Botrytis cinerea employs cell wall degrading enzymes especially the endopolygalacturonases to degrade components that constitute the cell wall (Elad, 1997; Reignault *et al.*, 2008). Deletion of genes (*Bcpg1* and *Bcpg2*) encoding these proteins resulted in reduced virulence of *B. cinerea* on tomato and bean leaves (ten Have *et al.*, 1998; Kars *et al.*, 2005a). Plants produce proteins referred to as polygalacturonase-inhibiting proteins (PGIPs) to counteract the effects of endopolygalacturonases (De Lorenzo *et al.*, 2001). Genes encoding these proteins, *AtPGIP1* (At5g06860) and *AtPGIP2* (At5g06870) were significantly up-regulated of which *AtPGIP1* was up-regulated as early as 12 hrs. *AtPGIP1* and *AtPGIP2* genes were also induced in response to *A. brassicicola* (Schenk *et al.*, 2003) which supports the supposition that their protein products play a significant role in host resistance to these pathogens (Ferrari *et al.*, 2003). In addition to inhibiting endopolygalacturonase activity, cell wall reinforcement is another early response that precedes pathogen recognition. A number of genes encoding enzymes involved in this process such as hydroxyproline rich glycoprotein and peroxidases were significantly up-regulated. Also up-regulated were genes associated with cell wall synthesis such as cellulose synthases and xyloglucosyl transferases. The up-regulation of genes involved in cell wall reinforcement and synthesis is a clear demonstration of the importance of the cell wall in resistance of *Arabidopsis* to *B. cinerea*. These genes provide good candidates for enhancing host resistance in susceptible plants.

3.3.2 JA/ET-dependent signalling in host resistance

In *Arabidopsis*, local and systemic resistance has been shown to be mediated by the JA/ET-dependent signalling pathways (Glazebrook, 2001). Genes encoding enzymes involved in the biosynthesis of these two hormones; lipoxygenase, *AOC* and *AOS* necessary for the biosynthesis of JA and ACC necessary for the biosynthesis of ET were significantly up-regulated. Exogenous application of these two signalling compounds results in induction of *PDF1.2*, a marker for this signalling pathway (Penninckx *et al.*, 1996). In the experiments reported here, this defensin was up-regulation which possibly confirms the presence of the two signalling compounds. Other up-regulated defensins included *PDF1.1*, *PDF1.2b*, *PDF1.2c*, *PDF1.3* and *PDF1.5*. Defensins are known to have antifungal activity, so could be key defence of the plant to produce family of them. Pathogenesis-related proteins, *PR3* (basic endochitinase B) and *PR4* (hevein like protein) were also up-regulated. Like *PDF1.2*, these genes are used to monitor the JA-dependent defence responses (Kunkel and Brooks, 2002).

Another gene induced by ET and JA is *ERF1*. This gene is known to also be induced following infection by *B. cinerea* (Berrocal-Lobo *et al.*, 2002; Lorenzo *et al.*, 2003). Constitutive expression of this gene in *Arabidopsis* confers resistance to *B. cinerea* and other necrotrophic pathogens (Berrocal-Lobo *et al.*, 2002). A study by Lorenzo *et al.* (2003) demonstrated that *ERF1* differentially regulates the expression of genes involved in defence against pathogens however, this same group of genes was shown to be repressed by an MYC transcription factor, *JIN1*. In this study *ERF1* was significantly up-regulated while *JIN1* was down-regulated even though this down-regulation was not considered significant due to variability in expression of this gene.

A number of other genes encoding *PR* proteins that are known to be induced by JA such as chitinases, glycosyl hydrolases, peroxidases and proteinase inhibitors were significantly up-regulated. Many of these proteins show strong antifungal activity and are directed towards structures of the fungus like the cell wall components glucan and chitin which are attacked by *PR2* and chitinases respectively (Fritig *et al.*, 1998; Odjakova and Hadjiivanova, 2001). Chitinases and osmotin from grapevine have been shown to inhibit *in vitro* growth of *B. cinerea* while enhancing

peroxidase activity enhanced disease resistance in transgenic plants (Kazan *et al.*, 1998). Over-expression of the LTP AceAMP1 from onion also increased resistance of scented geranium to *B. cinerea*. Proteinase inhibitors have also been shown to inhibit growth of *B. cinerea*, *Fusarium solani* f.sp. *pisi* and *A. brassicicola* (Lorito *et al.*, 1994; Joshi *et al.*, 1999; Heath, 2000). The up-regulation of these genes especially a multitude of glycosyl hydrolases and proteinase inhibitors suggests that engineering these genes especially by enhancing their activity in susceptible plants may improve their resistance to *B. cinerea*. Array data backs up importance of JA and ET signalling in defence against *B. cinerea*. It seems to be a response close to the lesion which suggests that strategies to activate JA/ET signalling earlier (in tissue further away from the lesion) could be an effective approach to decreasing susceptibility to *B. cinerea*.

3.3.3 SA-dependent signalling in host resistance

Salicylic acid has also been implicated in mediation of *Arabidopsis* defence responses against *B. cinerea* especially those required for arresting lesion development (Ferrari *et al.*, 2003). This signalling molecule is synthesized from phenylalanine in a reaction catalyzed by phenylalanine lyase (*PAL1* (*At2g37040*), *PAL2* (*At3g53260*), and *PAL3* (*At5g04230*)) (PAL pathway) or from chorismate in a reaction catalyzed by isochorisamte synthase (*ICS1* or *SID2* (*At1g74710*)) (ICS pathway). All the three genes in the PAL pathway are expressed at high levels in roots, *PAL1* and *PAL2* are expressed in shoots and only *PAL1* is expressed in leaves (Liang *et al.*, 1998). Recently Ferrari *et al.* (2003) demonstrated that SA synthesized via the PAL and not the ICS pathway was required for these defence responses. In this study, an up-regulation of *PAL1* and *PAL2* was observed in the spatial experiment. Although the up-regulation of *PAL1* was not deemed significant because of variability in expression, it recorded a 2-fold induction on average. *PAL2* on the other hand was significantly up-regulated more than 2-fold both close and away from the lesion while *PAL3* demonstrated slight increases in expression in the two experiments. The lack of expression of *PAL3* may be because leaves where it is known not to be expressed (Liang *et al.*, 1998) were used in the two experiments. Like *PAL3*, *ICS1* did not increase in expression in the two experiments. As Ferrari *et al.* (2003) suggested,

the PAL pathway seems to be essential in production of SA necessary for impeding *B. cinerea* development.

Studies have demonstrated that the SA-dependent signalling pathway may negatively antagonize the JA-dependent signalling pathway (Pieterse *et al.*, 2001a; Kunkel and Brooks, 2002; Glazebrook, 2005) yet this pathway has been shown to be very essential for mediating resistance responses to necrotrophs including *B. cinerea* (Thomma *et al.*, 1998; Vijayan *et al.*, 1998; Norman-Setterblad *et al.*, 2000). In *N. benthamiana*, El Oirdi and Bouarab (2007) demonstrated that *B. cinerea* exploits this negative crosstalk by up-regulating genes (*EDS1* and *SGT1*) involved in SA signalling. In this study, *EDS1*, *EDS5*, *PAD4* and *SID1* whose encoded proteins are essential components of the SA-dependent signalling pathway for disease resistance in *Arabidopsis* were not significantly up-regulated in both experiments. *EDS5* acts downstream of *EDS1* and *PAD4* in SA signalling (Kunkel and Brooks, 2002; Nawrath *et al.*, 2002; Glazebrook *et al.*, 2003). This implies *B. cinerea* possibly does not rely on this kind of antagonism to promote host colonization in *Arabidopsis* but may rely on other mechanisms. An example is the induction of genes encoding patatins (*At2g26560* (*AtPLP2*) and *At3g54950* (*AtPLP7*)). Camera and associates (2005) demonstrated that these genes are induced following infection by *B. cinerea* as observed in the two microarray experiments and proteins encoded by them especially *AtPLP2* facilitate host colonization of *B. cinerea* in *Arabidopsis*.

3.3.4 MPK4 may be more important in mediating *Arabidopsis* responses to *B. cinerea* than WRKY70

The WRKY70 transcription factor was shown to activate SA-inducible but repress JA-responsive genes even though this effect was not attributed to changes in levels of SA or JA (Li *et al.*, 2004, 2006). AbuQamar and associates (2006) recently reported the induction of *WRKY70* by almost 4-fold following infection by *B. cinerea*. They also demonstrated that mutants altered in this gene displayed enhanced susceptibility to *B. cinerea*. In contrast, there was almost no change in abundance of this gene in all replicates in the two experiments. The fact that this trend was observed in each of the replicates confirms that *WRKY70* does not possibly play a significant

role in this interaction most likely to this strain of *B. cinerea*. Unlike WRKY70, the MPK4-mediated signalling pathway represses SA-dependent disease resistance but activates JA-dependent disease resistance (Petersen *et al.*, 2000; Brodersen *et al.*, 2006).

The gene (*At4g01370*) encoding MPK4 was significantly induced in the spatial experiment (6-12 mm) but not the temporal experiment an indication that it is either expressed at low levels by 24 hrs or it displays restricted expression. The first supposition is supported by the expression of WRKY33 by 24 hrs after infection. This protein was recently shown by Andreasson and associates (2005) that it is a downstream component of the MPK4-mediated signalling pathway an indication that *MPK4* may be active. Recent studies by Zheng *et al.* (2006) also demonstrated that *WRKY33* is required for resistance to necrotrophic fungal pathogens. Put together, these results suggest that the MPK4 may be more important in mediating *Arabidopsis* responses to *B. cinerea* than WRKY70 in this interaction. It is necessary to elaborate the role of these two proteins in *Arabidopsis* resistance to this strain of *B. cinerea*.

3.3.5 Secondary metabolites play an important role in host resistance

Secondary metabolites especially the phytoalexins have been shown to play a significant role in impeding development of various pathogens including *B. cinerea* (Sbaghi *et al.*, 1995; Elad, 1997; Kliebenstein *et al.*, 2005). In this study, genes encoding enzymes involved in the synthesis of tryptophan were up-regulation an indication that tryptophan may be more important in the synthesis of secondary metabolites important in impeding development of *B. cinerea* in *Arabidopsis* than tyrosine and phenylalanine. Tryptophan is the precursor of camalexin, a phytoalexin shown to limit development of *B. cinerea* in *Arabidopsis*. In addition to camalexin, another class of compounds that may have a role in *Arabidopsis* resistance to *B. cinerea* are the terpenoids as a number of genes encoding enzymes involved in their biosynthesis such as the terpene synthases were up-regulated. This provides another avenue for testing.

A possible role for IAA does exist as genes encoding nitrilases, enzymes that mediate IAA biosynthesis from indole-3-acetonitrile (IAN) were up-regulated. Cluster analysis also demonstrated that gene expression profiles induced by IAA were very close to those induced by *B. cinerea* and secondly IAA up-regulated a number of genes up-regulated by *B. cinerea*. All these indicate a possible presence of this hormone in this interaction. Although up-regulation of nitrilases encoding genes supports IAA origin from the host, it is also possible that it originated from the pathogen. Recent studies by Maor and associates (2004) demonstrated that *Colletotrichum gloeosporioides* f.sp. *aeschynomene* synthesizes IAA by utilizing tryptophan and IAM both of which seem to be readily available in this interaction. This kind of mechanism has not been reported in *B. cinerea* pathogenicity. If it was present, could it be beneficial to the pathogen? Yes, this answer is based on two studies by Ek *et al.* (1983) and Shinshi *et al.* (1987). Ek *et al.* (1983) demonstrated that pathogen virulence had a direct correlation with levels of IAA which suggested positive function of this hormone in the infection process while Shinshi *et al.* (1987) reported that high concentrations may suppress expression of plant defence genes.

But because nitrilase genes were up-regulated, the argument that IAA could have been produced by the host for its benefit overrules the above argument. This supposition is supported by evidence from these studies. High concentrations of IAA were found to inhibit the HR (Jouanneau *et al.*, 1991; Robinette and Matthysse, 1990). The HR has been shown to be very beneficial in host colonization by *B. cinerea* (Govrin and Levine, 2000). Studies by Ueno *et al.* (2004) also demonstrated that infection of barley leaves pre-treated with IAA and other indolic-related compounds (tryptamine and tryptophan) resulted in reduced development of the rice blast fungus, *M. grisea*. They postulated that indolic-related substances seem to act by enhancing activity of enzymes involved in resistance such as PAL, chitinase and peroxidase as the level of these enzymes increased in pre-treated leaves before inoculation. A study by Llorentea *et al.* (2008) has recently demonstrated that *Arabidopsis* auxin signalling mutants *axr1*, *axr2*, and *axr6* that have defects in the auxin-stimulated SCF (Skp1-Cullin-F-box) ubiquitination pathway exhibited increased susceptibility to *B. cinerea* and another necrotroph *P. cucumerina*. This study suggested that auxin signalling may be essential in host resistance against necrotrophs.

Although auxins seem to promote host resistance to fungal pathogens, some studies have shown that they may also be involved in promoting host susceptibility to bacterial diseases. For instance, Navarro and associates (2006) showed that negative regulation of mRNAs for the F-box auxin receptors by a plant microRNA induced by a flagellin-derived peptide resulted in restricted growth of *P. syringae*. Most strains of *P. syringae* have been shown to synthesize high levels of IAA yet infecting *Arabidopsis* with the virulent *P. syringae* pv. *tomato* strain triggers increased levels of IAA (Glickmann *et al.*, 1998). Navarro *et al.* (2006) also showed in their study that application of the auxin analog 2,4-dichlorophenoxyacetic acid to *Arabidopsis* exogenously resulted in enhanced disease symptoms after infection with *P. syringae* pv. *tomato*. Put together, these studies demonstrate that bacterial pathogens seem to employ auxins in enhancing host susceptibility to these pathogens. Therefore, auxins seem to have contrasting roles in host-pathogen interactions.

3.3.6 Antioxidants are important in host resistance

Govrin and Levine (2000) demonstrated that the HR which is orchestrated by the accumulation of AOS (oxidative burst) promotes host colonization by *B. cinerea*. Studies by Tenberge *et al.* (2002) and Schouten *et al.* (2002) later confirmed that *B. cinerea* produces AOS whose main aim may be to accelerate maceration of host cells. A major part of the plant expression response was shown to be activated by AOS. Examples of genes activated by AOS included those encoding catalase, peroxidases and GSTs. All these proteins are known to protect host cells against AOS. For instance, catalase mediates the breakdown of H_2O_2 by converting it into molecular oxygen and water (Willekens *et al.*, 1997) while GSTs are involved in the biotransformation and detoxification of AOS and many other xenobiotic substances (Marrs, 1996; Coleman *et al.*, 1997). This indicates that most of the genes induced by *B. cinerea* may be encoding proteins involved in the protection against AOS. Therefore, combating effects of AOS in plants could be another major area for efforts to decrease susceptibility.

3.3.7 What could be the role of ABA in this interaction?

Abscisic acid (ABA) is a phytohormone known to play various roles during plant development including host responses to various environmental stresses (Finkelstein and Gibson, 2002; Seo and Koshiba, 2002). There is evidence that suggests that this hormone is significantly involved in the interaction between plants and pathogens since its endogenous levels have been observed to increase in response to infection with viruses, bacteria, and fungi (Whenham *et al.*, 1986; Kettner and Dörffling, 1995). The role of ABA in plant resistance seems to be conflicting; for instance, exogenous application prior to inoculation has been shown to enhance host susceptibility to pathogens such as *Erysiphe graminis* f.sp. *hordei* on barley, *Phytophthora megasperma* f.sp. *glycinea* on soybean and *B. cinerea*, *P. syringae* pv. *tomato* and *Peronospora parasitica* on *Arabidopsis* (Edwards, 1983; Ward *et al.*, 1989; Audenaert *et al.*, 2002; Mohr and Cahill, 2003). Audenaert and associates (2002) also demonstrated that *sitiens*, a mutant deficient in ABA production was more resistant to *B. cinerea*, a phenotype they lost on application of ABA which stressed the role of this hormone in enhancing host susceptibility to pathogens.

However, recent studies have demonstrated that ABA may as well enhance host resistance towards pathogens. For instance, Ton and Mauch-Mani (2004) reported that ABA primes for enhanced callose deposition. Adie *et al.* (2007) also reported that ABA appears to enhance resistance of *Arabidopsis* to *P. irregulare* as wild-type plants which display increased levels of ABA are more resistant than ABA biosynthesis and insensitive mutants. In this study, three observations were made; genes involved in ABA biosynthesis were up-regulated; the ABA responsive element (ABRE) was over-represented in promoters of significantly up-regulated genes and genes up-regulated by *B. cinerea* and ABA appeared to be co-regulated. All this indicated a possible presence of ABA which may be originating from the pathogen (Kettner and Dörffling, 1995; Siewers *et al.*, 2004, 2006) or the host. Biosynthesis of ABA by the host could be a side effect of infection, an active response of the host or a manipulation of the host by the pathogen. This second supposition is supported by studies of Torres-Zabala *et al.* (2007) who demonstrated that effector-mediated manipulation of ABA biosynthesis and signalling could be the major virulence strategy employed by *P. syringae* pv. *tomato* to suppress host defence responses.

3.3.8 Kinases at the helm of host resistance

Protein kinases regulate many classes of proteins through phosphorylation resulting in activation of many cellular processes (Morris, 2001; Romeis, 2001). For instance, phosphorylation of transcription factors may result in activation of gene expression. A number of genes encoding protein kinases were up-regulated after infection of *Arabidopsis* with *B. cinerea*. One of the up-regulated kinase encoding genes was *OXI1* (*At3g25250*). This gene has been shown to be induced in response to many stimuli that generate H_2O_2 (Rentel *et al.*, 2004) which is also produced in this interaction. Knock-out mutants of *OXI1* have also been shown not to be more susceptible to *B. cinerea* infection an indication that it may not play a significant role in resistance of *Arabidopsis* to *B. cinerea*. However, a recent study by Veronese *et al.* (2006) demonstrated that *BIK1* (*At2g39660*) another kinase encoding gene up-regulated in this study has a direct role in resistance of *Arabidopsis* to *B. cinerea*. Inactivation of this gene resulted in mutants highly susceptible to necrotrophic fungal pathogens.

Examples of the other up-regulated kinase genes include *At2g18170* (*AtMPK7*) and *At1g21250* (*WAK1*). At2g18170 (AtMPK7) is a MAP kinase similar to Os06g49430 in rice. Exposure of rice plants to JA, ABA, H_2O_2, salt, drought and infection by *M. grisea* induced expression of *Os06g49430* (Jeong *et al.*, 2006; Reyna and Yang, 2006). *WAK1* is a wall-associated kinase, it is also induced by pathogens (e.g. *P. syringae* and *A. brassicicola*), ET, MeJA and SA (He *et al.*, 1998; Maleck *et al.*, 2000; Schenk *et al.*, 2000). Recently it was shown that this kinase interacts with cell wall pectins in a calcium-induced conformation an action that may be very important in host-pathogen interactions (Decreux and Messiaen, 2005). The fact that these kinases were up-regulated suggests that possibly some of the other up-regulated kinases may also have important roles in resistance of *Arabidopsis* to *B. cinerea* hence the necessity to investigate their role in resistance through reverse genetics.

3.3.9 Repression of the photosynthetic pathway

One of the salient observations was the down-regulation of genes encoding enzymes involved in the photosynthetic pathway. This down-regulation appears to be a general response to pathogen infection and has been reported in a number of compatible as well as incompatible interactions. For instance, rubisco was down-regulated in *Arabidopsis* leaves infected with *Albugo candida* however, in this same experiment, an increase in activity of invertases which was confined in areas invaded by the fungus was also observed (Chou *et al.*, 2000). Repression of photosynthetic genes hasalso been reported in nonhost interactions such as the one involving *Blumeria graminis* f.sp *hodei*, a nonhost pathogen to *Arabidopsis* (Zimmerli *et al.*, 2004). Therefore, down-regulation of photosynthetic genes appears to be a response to infection by all groups of pathogens however; the magnitude of gene regulation may vary depending on the attacking pathogen.

The most probable reason for this down-regulation could be due to elevated levels of soluble carbohydrates due to increased invertase activity (Chou *et al.*, 2000). When carbohydrate levels exceed the plants capacity to metabolize, a signal transduction cascade is initiated leading to repression of photosynthetic gene expression (Chou *et al.*, 2000). This line of explanation has been supported by many studies. For instance, addition of sugars to external media has been shown to down-regulate the expression of both nuclear and plastidic genes for photosythetic components (Pego *et al.*, 2000). Repression of rubisco was observed in leaf discs floated in a solution of sucrose/glucose while in another experiment, repression of seven promoters of genes encoding photosynthetic components was observed after the external media was supplied with various sugars (Jang and Sheen, 1994). Fascinatingly, two genes encoding invertases (*At1g62660* and *At3g13790*) and three encoding fructokinase (*At1g50390*, *At2g31390* and *At3g59480*) all of which are involved in sucrose degradation were significantly up-regulated in the two experiments (Appendix A.1, A.3, B.1 and B.3).

3.3.10 Biological significance of up-regulated genes

Although infection of *Arabidopsis* by *B. cinerea* resulted in up-regulation of a multitude of genes, analysis of mutants altered in some of these genes did not give a clearly defined phenotype. Similar observations were also made by AbuQamar *et al.* (2006). They postulated that some of the genes may not have a direct role in resistance to *B. cinerea* but are induced through secondary effects. Secondly, functional redundancy among the genes induced by *B. cinerea* which belong to the same multi gene family may also lead to weaker or lack disease phenotype (AbuQamar *et al.*, 2006). One of the ways to get around this problem is either to use RNAi lines designed to knock-out the whole family of genes. The second option is to overexpress the genes of interest and test for increased resistance.

3.3.11 Spatial expression of *Arabidopsis* genes induced by *B. cinerea*

Studies by Kliebenstein *et al.* (2005) and Ferrari *et al.* (2003) demonstrated the possibility of spatial expression of metabolites in *Arabidopsis* following infection by *B. cinerea*. In this study it was observed that in addition to temporal expression, *Arabidopsis* genes induced following infection by *B. cinerea* were spatially expressed as tissue close and away from the developing lesion responded differently to the pathogen. This observation indicated that some type of signal may be emanating from the pathogen which the plant is responding to. This hypothesis is supported by the significant over-representation of the ABA responsive element close to the developing lesion yet in the tissues away it was not significant. It is worth performing more replicates of this experiment and also over a time course to ascertain some of these changes. Secondly, it will be interesting to look at much higher spatial resolution through application of laser microdissection.

3.4 Conclusion

This study employed microarrays to identify genes involved in *Arabidopsis* resistance to *B. cinerea*. A number of genes that have been identified in various studies but not on a transcriptomic level and shown to be involved in *Arabidopsis* resistance to *B. cinerea* were also induced in this study which demonstrates the usefulness of microarrays. Biotrophic pathogens follow a gene-for-gene resistance pattern in which an *avr* gene product is recognized by an *R* gene product. Necrotrophs on the other hand do not to follow this type of resistance pattern but host resistance to these pathogens involves an array of pathogen-inducible genes. In agreement with this proposition, infection of *Arabidopsis* with *B. cinerea* induced a multitude of genes. Some of these genes were induced in space and time. Although so many genes were induced, functional studies resulted in only one mild phenotype an indication of redundancy. Using RNAi lines designed to knock-out the whole family of genes or overexpressing the genes of interest can help get around this problem.

This study has highlighted several genes and/or processes which may be important in determining susceptibility to *B. cinerea* and which should be the focus of further study. For example, among the secondary metabolites, terpenoids may have a significant role in host resistance. Besides ET, JA and SA, other phytohormones that seem to play significant roles in this interaction are ABA and IAA. The role of ABA in host resistance needs to be elucidated with ABA-deficient *Arabidopsis* mutants. Functional studies of *B. cinerea* ABA-deficient mutants will also enhance our understanding on whether *B. cinerea* employs ABA as a pathogenicity factor or ABA is produced in the host as a response to secondary abiotic effects of *B. cinerea* infection. Similarly use of mutants unable to produce IAA will enhance our understanding on the role of this hormone in this interaction. The WRKY70 transcription factor was shown to be important in host resistance to *B. cinerea* however, in this study, the MPK4 appears to be more important in mediating *Arabidopsis* resistance to *B. cinerea*.

All genes identified in this study are potential candidates for functional genomic studies, however, those encoding kinases, transcription factors, terpenoids and G proteins provide immediate candidates. ABC transporters have very important roles in both host resistance and pathogen virulence, it is therefore important that

the phenotype displayed by *PDR12* is confirmed but also investigate the role of other ABC transporters up-regulated in this interaction. It is also worth investigating the role of the MPK4- and WRKY-mediated signalling in *Arabidopsis* resistance to *B. cinerea* especially to this strain of *B. cinerea*. This will not only enlighten our understanding of the role of the two signalling pathways but also demonstrate if some resistance mechanisms are strain specific. The spatial experiment has demonstrated that some of the *Arabidopsis* genes responsive to *B. cinerea* are spatially expressed. It is therefore worth performing multiple replicates of this experiment over a time course to determine genes expressed in both time and space. Resistance factors expressed close to the lesion such as the terpenoids may be very valuable in impeding *B. cinerea* development hence provide good candidates for breeding programs.

Chapter 4

Proteomic Analysis of *Arabidopsis thaliana* - *Botrytis cinerea* Interaction

4.1 Rationale

Although enormous information is generated from transcriptome expression profiling studies, this information is not enough to elucidate the functioning of biological systems especially at a molecular level (Patterson, 2004). This is because, these studies only provide information about the abundance of mRNA which is only the first step in long sequence of events that lead to the formation of proteins, the main working molecules of cells (Lodish *et al.*, 2004). Upon formation, mRNA undergoes a number of post-transcriptional modifications such as addition of a *5'cap*, *a poly(A) tail* and *splicing* to remove introns. Through alternative splicing (Lareau *et al.*, 2004; Stamm *et al.*, 2005; Reddy, 2007), a molecular process that is very common in eukaryotes, a single mRNA may generate dozens of different mRNA isoforms all giving rise to different proteins. In fact the average number of protein forms estimated per gene were predicted to be one or two in bacteria, three in yeast, and three or more in humans (Wilkins *et al.*, 1996; Krishna and Wold, 1993).

Upon translation, proteins undergo a number of post-translation modifications either in form of covalent modifications or proteolytic cleavage at specific amino acid residues (Blom *et al.*, 2004; Gomord and Faye, 2004). Covalent modifications which are reversible events are important in determining the state of activity of a protein (Simpson, 2003). Examples include acetylation, carboxylation, glycosylation, hydroxylation, methylation, nitrosylation, phosphorylation, transamidation, ubiquitination and many others (Mann and Jensen, 2003; Blom *et al.*, 2004; Kwon *et al.*, 2006). Proteolytic cleavage on the other hand is irreversible; it results in a truncated form of a protein. Truncation may occur at the amino or carboxy terminal end or may take place internally. These truncations alter the state of activity of proteins; for instance, certain proteolytic enzymes are produced as inactive precursors (zymogens), which must be cleaved to generate an active enzyme. Examples include prosystemin which undergoes proteolytic cleavage to release systemin in tomato (Ryan, 2000; Gatehouse, 2002) and storage proteins in developing and germinating dicotyledonous seeds (Müntz, 1996).

Post-translational modifications are therefore very important in signal transduction as they are important in determining the function of various proteins. Post-transcriptional modifications determine the number of gene products to expect while

post-translational modifications affect their intrinsic biological activity and subcellular localization however, both these processes can not be analyzed by genomic approaches. Because of these modifications, the number of expressed transcripts is not always suggestive of the corresponding translated proteins at either steady state or in response to a stimulus (Patterson, 2004). This supposition is supported by many studies which have shown a poor correlation between the transcribed mRNA and expected translation products (Gygi *et al.*, 1999; Ideker *et al.*, 2001; Kern *et al.*, 2003). The main objective of this study was to determine whether levels of protein corresponding to the genes up- or down-regulated in the microarray experiment do increase or reduce respectively following infection of *Arabidopsis* with *B. cinerea*.

Two approaches may be used to achieve the above objective. In the first approach, full length cDNAs corresponding to some of the genes significantly up-regulated after infection of *Arabidopsis* with *B. cinerea* can be cloned into bacterial expression vectors. The resulting recombinant proteins are then used to generate polyclonal antibodies. These can then be tested to ensure that they only cross-react against the selected protein and not against other plant proteins. Protein levels corresponding to the selected genes can then be determined over a time course of *B. cinerea* infection using ELISA if the antisera do not cross react with other plant proteins or western blotting if there is some non-specific cross reactivity.

The second approach is to profile protein expression on a global scale. A number of methods have been developed for the purpose of identifying expressed proteins and can be grouped into gel- and non-gel-based methods. Gel-based methods include two dimension sodium dodecyl sulphate polyacrylamide gel electrophoresis (2D SDS-PAGE) (Klose, 1975; Gorg *et al.*, 1999) and two dimension differential gel electrophoresis (2D DIGE) (Patton and Beechem, 2002; Peck, 2005). In gel based methods, first dimension resolution of proteins is based on their isoelectric point (pI) while second dimension on their molecular mass (MW). These methods suffer from a number of shortcomings; they are not particularly good at resolving lower abundance, low molecular weight, very large, hydrophobic or membrane proteins and those with extreme pI values (Yan and Chen, 2005; Peck, 2005; Hamler *et al.*, 2004). The introduction of 2D DIGE minimized the problem of gel-to-gel variation which is very common in 2D SDS-PAGE (Patton and Beechem, 2002).

In non-gel methods, protein identification is either based on isotope labeling or liquid-phase separation. In isotope labeling, quantification is performed by differential incorporation of stable isotopes. This may be performed either *in vivo* such as in metabolic labeling and stable isotope labeling with amino acids in cell culture (SILAC) or *in vitro* such as in isotope-coded affinity tags (ICAT) and isobaric tags for relative and absolute quantification (iTRAQ) (Yan and Chen, 2005). Liquid phase separation methods are based on the principle of chromatography and three most commonly used types are thin layer, paper and column chromatography (Luo *et al.*, 1998). However, column chromatography also referred to as fractionation or purification chromatography is the most powerful of all.

Column chromatography separation methods include gel filtration, affinity and ion exchange chromatography (Mann *et al.*, 2001; Zhu *et al.*, 2003). Ion exchange chromatography can be employed to separate proteins in two dimension; in the first dimension, proteins are separated based on their pI and this procedure referred to as chromatofocusing. In this procedure, proteins are bound to an anion exchanger and then eluted by continuous decrease of the buffer pH so that proteins elute in the order of their pIs. This is achieved by using amphoteric buffers titrated to the lower pH to generate a more linear gradient. By changing the pH of the buffer, the pH range used for separation can be controlled. Chromatofocusing is fast, has a high resolving power and combines unique selectivity with the ability to retain proteins in their native state.

In the second dimension, proteins are separated based on their hydrophobicity. Reversible hydrophobic interactions occur between amino acid side chains of the protein with the hydrophobic surface of the stationary phase of the column. The stationary phase is non-polar while the mobile phase is composed of an organic solvent (acetonitrile, ACN) and an aqueous buffer (water). An organic solvent acts as a modifier, it is added to the aqueous mobile phase to lower its polarity so that proteins may elute from the column. Proteins elute according to their degree of hydrophobicity, which is calculated by the percentage content of non-polar amino acids. ACN is preferred to other organic solvents because it is less viscous, hence results in less back pressure and high efficiency and is also UV transparent which is important because most reversed-phase separations are monitored below 220 nm for

optimal detection of peptides (Simpson, 2003).

An acid (e.g. Trifluoroacetic acid, TFA) is added to the organic solvent and aqueous buffer to lower their pH. This is important in reverse-phase separations because high pH cause cause tailing of peaks but mostly may cause dissolution of silica-based reverse-phase packings leading to the collapse of the packed bed. Lowering of the pH also improves sample solubility. TFA is preferred to other acids because it is volatile hence easily removed by lyophilization and also has a low absorption with detection wavelength for peptides and protein. Because it has an absorbance in water different from that in ACN, the concentration of TFA in ACN is made at 85-90% the concentration in water (Simpson, 2003). This avoids a baseline shift during gradient formation. Separation of proteins using liquid chromatography (LC) enables crude protein extracts to be analyzed with a few purification steps. This increases reproducibility and allows better comparison of protein patterns (Simpson, 2003). Because high initial amounts of proteins are injected into the system, this also increases the possibility of resolving low abundant proteins. A number of systems based on the two dimension liquid separation principle have been developed. An example of such a system is the ProteomeLab™ PF2D protein fractionation system developed by Beckman Coulter, Inc. USA (www.beckmancoulter.com). To study the infected proteome of *Arabidopsis*, we employed the classical 2D SDS-PAGE and the 2D LC system, ProteomeLab™ PF2D.

4.2 Results

4.2.1 2D SDS-PAGE analysis

Arabidopsis plants were grown under the same conditions as those used in the microarray experiment. Leaves were inoculated with *B. cinerea* and harvested after 6, 12 and 24 hpi. Mock treatments were conducted with half-strength grape juice without *B. cinerea* spores. The inclusion of the 6 hr time point was based on microarray results which showed that a considerable number of genes were up-regulated by 12 hrs. This indicated that there could possibly be genes up- or down-regulated at earlier time points. The increase or decrease in abundance of their protein products could be determined by 6 hrs. The experiment was biologically replicated 5 times

with tissue for each replicate grown and inoculated on a separate dates. All harvested tissue was kept at -70°C until protein extraction. To minimize variability, protein from all harvested tissue for all replications was extracted at the same time using the same extraction buffer.

To determine the optimum conditions for the 2D SDS-PAGE, a pilot experiment was conducted in which total protein was extracted from two samples (infected and uninfected leaf tissue) and separated on broad range (pH 3-10) IPG strips for the first dimension and a 12% linear poly-acrylamide gel for the second dimension. The majority of the protein spots were detected in the centre of the gel, indicating that a narrower pH range for the IPG strip was necessary for increased resolution. In addition, proteins were well separated from top to bottom by their molecular weight. Therefore, strips with pH 4-7 range and a 12% linear polyacrylamide gel were used in all subsequent experiments. The experiment was carried out over a time series (6, 12 and 24 hrs) and replicated 5 times. Two gels (mock and treated) were prepared for each replicate. To minimize gel-to-gel variability, gels (10) from each replicate were run concurrently. The Mini-PROTEAN® 3 Dodeca Cell (Bio-Rad, Laboratories Inc, Hercules, USA) which can run up to 12 gels was used. Technical replicates of infected protein samples were included in each run to make up 12 gels hence 36 in total. Because this system runs mini gels, strips of 7 cm strips and pH 4-7 as already determined were used.

The majority of protein spots separated very well except for a few which were around pI of 5 and MW of 45 kDa. However, the resolution of protein spots across all the 36 gels was highly reproducible (Fig. 4.1). Some proteins spots assumed to be basic proteins were condensed at the end in the pH 7 range (Fig. 4.1). All spots were matched by gel-to-gel comparison, using PDQuest software. Following background subtraction and automated spot detection, spots were manually matched across all gels in a match set. This alignment was based on a number of selected landmarks. Before subjecting spot intensities to quantitative analysis, they were normalized to remove variation that is not attributable to differential protein expression. This variation can be caused by a number of factors which include differences in sample preparation, loading, staining and imaging between gels. The built in normalization of total density in gel image was used; in this normalization

method, the raw quantities of each spot in a member gel, is divided by the total intensity value of all pixels in the image.

On average, 255 protein spots were detected in each of the gels. The low number of differentially expressed proteins identified was attributed to the size of gels (7 cm) and sensitivity of the dye used. Quantitative comparisons were performed between mock and infected samples in each of the time points. Protein spots whose abundance increased or decreased significantly (t-test, $P < 0.05$, n=5) after treatment of *Arabidopsis* leaf tissue with *B. cinerea* were selected. Based on the analysis, 17, 21 and 35 protein spots displayed significant changes in abundance between the mock and treated samples after 6 , 12 and 24 hpi respectively (Table. 4.1 and Fig. 4.1). This represents 6.7, 8.2 and 13,7% of the total number of resolved proteins respectively. Of the 17, 21 and 35 spots that displayed significant changes in abundance, 5 increased by more than 2 fold on average after 6 and 12 hrs while 6 increased by the same fold after 24 hrs (Table. 4.1). Four, five and thirteen protein spots displayed increases in abundances after 6, 12 and 24 hrs however, these increases were less than 2 fold. The number of protein spots that displayed significant reductions in abundance were 7, 11 and 15 after 6, 12 and 24 hrs respectively (Table. 4.2 and Fig. 4.1). This represents 2.7, 4.3 and 5.9% of the total number of resolved proteins respectively. Of the 7, 11 and 15 spots that displayed significant reductions in abundance, 3 reduced by more than 2 fold on average after 6 and 12 hrs while 7 reduced by the same fold after 24 hrs (Table. 4.2). Comparisons were also made across the time series. Among the up-regulated protein spots, none of them was up-regulated in all three time points however, 2 proteins spots were up-regulated between 6 and 12 hpi and 6 and 24 hpi while 3 protein spots were up-regulated between 12 and 24 hpi. Results are presented in Fig. 4.2A. On the contrary, 2 protein spots were down-regulated in all three time points while 1 protein spot was down-regulated between 12 and 24 hpi. No down-regulated protein spots were common between 6 and 12 hpi as well as 12 and 24 hpi (Fig. 4.2B). These results suggest that infection of *Arabidopsis* with *B. cinerea* results in significant changes in host response which can be observed at the proteome level. These changes possibly occur even before 6 hrs.

Table 4.1: *Arabidopsis* proteins that significantly increased in abundance after 6, 12 and 24 hrs following infection of *Arabidopsis* leaf tissue with *B. cinerea*. The experiment was replicated five times (Rep 1-5) and only protein spots that significantly increased in abundance are shown. Values of Rep 1-5 and average represent fold change, ± represents the standard error and SSP represents the standard spot number. The fold change was determined by dividing spot intensities of gels prepared from the infected sample with spot intensities of gels prepared from the mock treated sample.

SSP	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Mean
6 hpi						
7104	2.91	3.05	1.98	1.62	2.35	2.38±0.27
7005	1.40	4.88	2.29	1.22	1.80	2.32±0.67
9403	1.72	0.86	3.46	2.48	2.26	2.16±0.43
6003	2.39	1.52	2.13	2.00	2.29	2.07±0.15
8501	3.09	0.98	1.42	1.60	3.00	2.02±0.43
5002	0.92	1.64	2.48	2.47	2.07	1.92±0.29
3603	1.95	1.10	2.49	1.93	2.07	1.91±0.23
1901	1.12	1.15	2.36	1.39	1.20	1.44±0.23
3304	1.44	1.12	1.13	1.30	0.97	1.20±0.08
12 hpi						
1004	1.89	2.45	4.60	4.49	6.75	4.04±0.87
5001	5.93	2.67	2.11	5.12	3.01	3.77±0.74
6001	4.70	3.02	1.42	4.50	3.75	3.48±0.59
7301	2.91	1.50	1.22	6.44	1.36	2.69±0.99
7803	2.18	2.01	1.86	1.68	4.50	2.45±0.52
5808	1.39	2.42	2.18	1.15	2.43	1.91±0.27
3102	1.71	1.50	1.40	1.20	1.62	1.49±0.09
2603	1.26	1.88	1.45	1.54	1.11	1.45±0.13
2102	1.71	1.63	1.47	1.28	1.03	1.42±0.12
1503	1.33	1.02	1.13	1.38	1.30	1.23±0.07
24 hpi						
9205	5.83	3.12	5.25	2.13	7.39	4.75±0.95
1104	3.90	4.63	5.15	3.33	4.93	4.39±0.34
7102	2.85	1.67	1.84	5.35	2.58	2.86±0.66
5001	1.51	1.32	1.79	1.69	5.75	2.41±0.84
6903	1.73	5.81	1.53	1.34	1.14	2.31±0.88
3702	1.71	2.69	1.90	2.18	2.47	2.19±0.18
4401	1.91	1.54	1.26	3.14	1.49	1.87±0.33
3902	1.93	3.16	1.97	1.13	0.87	1.81±0.40
7801	1.00	2.20	1.21	2.03	2.20	1.73±0.26
4901	1.60	2.37	1.64	1.32	1.64	1.72±0.17
1905	1.49	2.66	1.51	0.76	1.79	1.64±0.31
7901	1.04	2.47	1.92	1.20	1.54	1.63±0.26
5202	1.50	1.08	2.04	2.12	1.36	1.62±0.20
2901	1.30	1.80	1.11	1.63	1.81	1.53±0.14
1005	1.74	1.58	1.40	1.21	1.57	1.50±0.09
2302	2.00	1.01	1.16	2.01	1.23	1.48±0.22
3502	1.08	1.78	1.27	1.20	1.55	1.38±0.13
7101	1.02	1.12	1.22	1.27	1.87	1.30±0.15
3401	1.44	1.56	1.15	0.99	1.16	1.26±0.10

Table 4.2: *Arabidopsis* proteins that significantly decreased in abundance after 6, 12 and 24 hrs following infection of *Arabidopsis* leaf tissue with *B. cinerea*. The experiment was replicated five times (Rep 1-5) and only protein spots that significantly decreased in abundance are shown. Values of Rep 1-5 and average represent fold change, ± represents the standard error and SSP represents the standard spot number. The fold change was determined by dividing spot intensities of gels prepared from the infected sample with spot intensities of gels prepared from the mock treated sample.

SSP	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Mean
6 hpi						
2502	0.74	1.38	0.46	0.78	0.27	0.73±0.19
6605	0.68	0.76	0.66	0.45	0.89	0.69±0.07
7002	0.38	0.37	0.52	1.21	0.41	0.58±0.16
5103	0.50	0.62	0.43	0.59	0.66	0.56±0.04
2001	0.57	0.77	0.32	0.35	0.19	0.44±0.10
8301	0.27	0.25	0.27	0.22	0.86	0.37±0.12
4001	0.13	0.45	0.15	0.28	0.25	0.25±0.06
12 hpi						
0004	0.95	0.66	0.87	0.81	0.85	0.83±0.05
1104	0.79	0.72	0.59	0.79	0.70	0.72±0.04
7801	0.84	0.90	0.70	0.54	0.51	0.70±0.08
7101	0.68	0.69	0.72	0.94	0.30	0.67±0.10
9301	0.67	0.49	0.91	0.80	0.44	0.66±0.09
4603	0.49	0.58	0.26	1.12	0.67	0.62±0.14
5104	0.48	0.41	0.80	0.91	0.42	0.60±0.10
6106	0.33	0.48	0.39	1.15	0.39	0.55±0.15
3501	0.18	0.43	0.43	0.62	0.20	0.37±0.08
2003	0.24	0.31	0.23	0.97	0.09	0.37±0.15
4001	0.25	0.22	0.32	0.32	0.13	0.25±0.04
24 hpi						
3101	0.64	0.58	0.57	1.40	0.73	0.79±0.16
1001	0.93	0.61	0.73	0.78	0.82	0.77±0.05
6401	0.87	0.69	0.67	0.83	0.76	0.76±0.04
4403	0.70	0.83	0.96	0.71	0.58	0.76±0.06
3601	1.16	0.79	0.67	0.48	0.56	0.73±0.12
3402	0.84	1.05	0.86	0.50	0.39	0.73±0.12
0001	1.26	0.57	0.45	0.37	0.56	0.64±0.16
2001	0.87	0.86	0.38	0.15	0.64	0.58±0.14
2603	0.75	0.97	0.46	0.05	0.08	0.46±0.18
0006	0.24	0.20	0.39	0.12	0.90	0.37±0.14
1002	0.49	0.24	0.15	0.48	0.31	0.33±0.07
7301	0.31	0.17	0.40	0.34	0.39	0.32±0.04
4104	0.08	0.78	0.31	0.29	0.09	0.31±0.13
2002	0.44	0.19	0.41	0.04	0.10	0.24±0.08
3001	0.51	0.16	0.22	0.01	0.09	0.20±0.09

Table 3.8: Functional categorization of genes in each of the 11 clusters based on biological process. The number and percentage represent the number and proportion of up- or down-regulated genes involved in a specific biological function (responsive genes) and the number and proportion of all genes in the genome involved in a similar biological function (whole genome). Only categories significantly over-represented are shown.

	Responsive genes		Whole genome		
Entity	Number	Percentage	Number	Percentage	P-value
Cluster 1					
Macromolecule metabolic process	85	29.8	4688	42.9	3.34E-03
Response to chemical stimulus	45	15.8	899	8.2	8.84E-03
Cluster 10					
Biosynthetic process	82	23.1	1500	13.8	3.41E-03
Cluster 11					
Photosynthesis	35	8.4	44	0.4	2.59E-26
Biosynthetic process	97	23.4	1485	13.7	1.23E-04
Carbon utilization	6	1.5	9	0.1	1.42E-03

Table 3.9: 6-mer elements representing known *cis* motifs that are over-represented in the 500 bp regions upstream of the ATG sites in clustered genes. Only clusters with significantly over-represented 6-mer elements are shown.

		Absolute number of oligomer		Number of sequences containing oligomer		
Motif name	Oligomer	Query set	Genomic set	Query set	Genomic set	P-value
Cluster 1						
TGACGT	TGA1	134	4805	113	4060	4.92E-07
ACGTCA	LS7	134	4805	113	4060	4.92E-07
ACGTGG	ABRE	128	5245	106	4214	6.13E-05
CACCAA	MYB	211	10156	171	8539	8.10E-03
CACGTG	G-box	184	7396	76	3097	1.00E-03
Cluster 2						
CACGTG	G-box	42	7396	16	3097	4.56E-03
Cluster 3						
CACGTG	G-box	54	7396	23	3097	5.18E-09
ACGTGG	ABRE	25	5245	22	4214	5.05E-06
TGACGT	TGA1	20	4805	15	4060	5.56E-03
ACGTCA	LS7	20	4805	15	4060	5.56E-03
Cluster 4						
CACGTG	G-box	124	7396	50	3097	5.28E-08
ACGTGG	ABRE	72	5245	53	4214	2.73E-05
TTGACT	W-box	63	13337	53	10320	2.96E-04
Cluster 5						
TTGACT	W-box	149	13337	102	10320	1.03E-09
ACGTAA	OCS	67	6633	57	5565	9.86E-06

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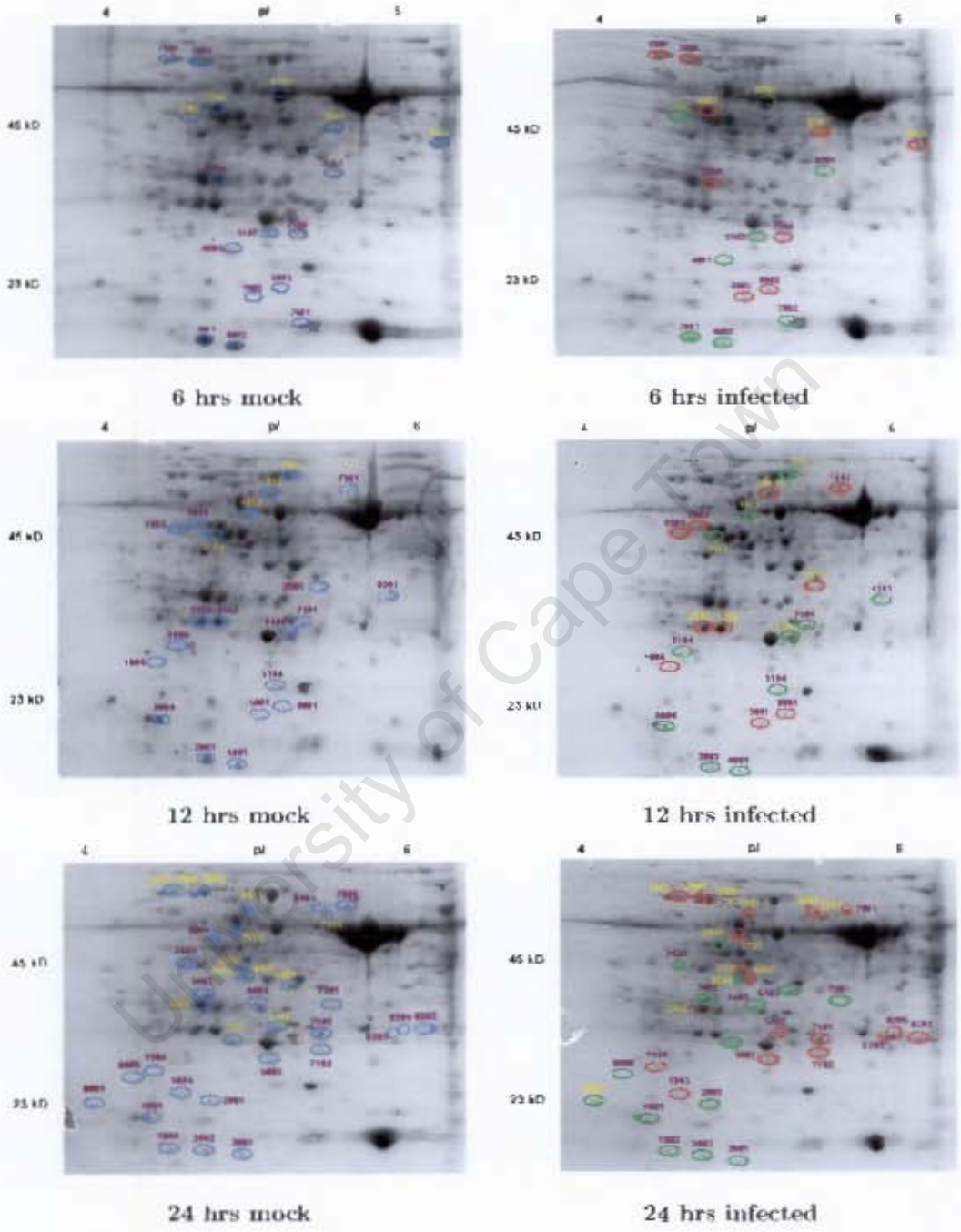
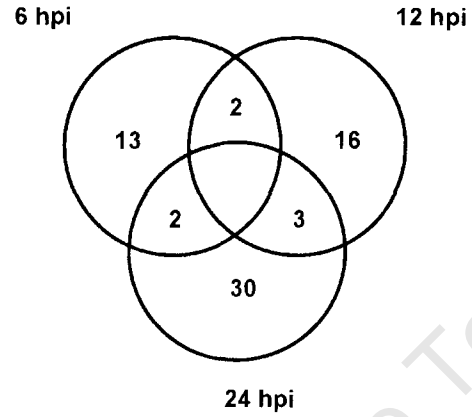
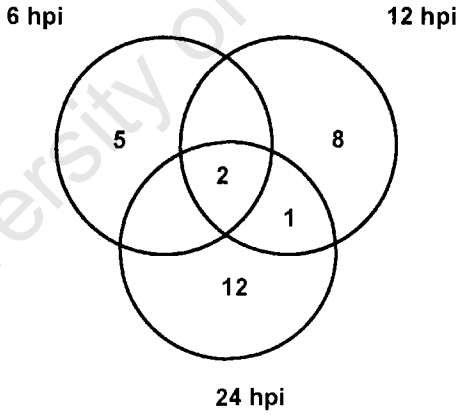


Figure 4.1: 2D SDS-PAGE images of proteins extracted from four-week *Arabidopsis* leaf tissue mock treated and infected with *B. cinerea*. The gels were stained with colloidal coomassie. The red and yellow circles in gels from treated samples represent protein spots that were significantly up- (red) and down-regulated (green) at the three time points. Corresponding spots have been highlighted in blue in gels prepared from mock treated samples. Gels from replicate 1 are presented for all time points. SSP numbers for corresponding spots in all time points are different because each time point was considered separately during analysis. Images for replicates 2-5 are presented in Appendix C.



A



B

Figure 4.2: Four week old *Arabidopsis* leaf tissue was infected with *B. cinerea* and proteins extracted and separated with 2D SDS-PAGE. The experiment was replicated five times. The number of protein spots significantly up-regulated (A) and down-regulated (B) are shown.

To determine the type of proteins that were increasing in abundance after infection, 4 protein spots (1104, 8205, 9202 and 9205) (Fig. 4.1) that significantly increased in abundance after 24 hrs were manually excised from gels prepared from total proteins extracted from *Arabidopsis* tissue infected with *B. cinerea* and harvested after 24 hrs. These spots were selected because they could easily be cut out without contamination with other spots. Preferably, protein spots whose abundance increases in the early time points such as 6 and 12 hrs would provide very good candidates for mass spectrometry identification however, the intensity of these spots was very low, hence it was envisaged that they would not give a good signal for identification. The excised spots were digested with trypsin and the peptides analyzed with a 4800 MALDI-TOF mass spectrometer (Applied Biosystems, Framingham, MA, USA). The peptide mass fingerprints generated were used to search the National Center for Biotechnology Information nonredundant (NCBInr) protein database found at http://www.ncbi.nlm.nih.gov using MASCOT software (http://www.matrixscience.com). Three of the four selected protein spots were successfully identified as At1g02930 (8505) and At4g02520 (9202 and 9205) all of which were glutathione S-transferases (GSTs) (Table. 4.3).

Gel images were compared with 2D SDS-PAGE gels publicly available on WORLD-2DPAGE (http://au.expasy.org/swiss-2dpage/) to determine if some of the protein spots that significantly increased or decreased in abundance could be identified in 2D SDS-PAGE experiments available publicly. Two spots, SSP 7002 (6 hrs infected) and SSP 7101 (24 hrs infected) (Fig. 4.1) were correlated. SSP 7002 is RuBisCO (At5g38410) while SSP 7101 is ascorbate peroxidase 1 (At1g07890). RuBisCO was significantly down-regulated while ascorbate peroxidase up-regulated.

Table 4.3: Proteins separated by 2D SDS-PAGE from *Arabidopsis* leaf tissue infected with *B. cinerea* and harvested after 24 hpi. Columns report the standard spot number (SSP), accession number (NCBInr database), gene locus, theoretical p*I* and MW and the description

SSP	Accession	Locus	p*I*	MW	Description
8205	15218640	At1g02930	5.8	23471	Glutathione S-transferase
9205	13194824	At4g02520	5.9	24114	Glutathione S-transferase
9202	13194824	At4g02520	6.1	24114	Glutathione S-transferase

4.2.2 2D liquid chromatography

To get around some of the limitations of the 2D SDS-PAGE technique, the 2D liquid chromatography technique which is applied in the ProteomeLab PF2D system (Beckman Coulter, Fullerton, CA) was used. The assumption was that using the 2D LC system, a better resolution would be realized. Tissue used for extracting total protein in this system was grown under the same conditions as tissue used in the previous experiments. Total protein was extracted with a lysis buffer composed of *n*-octylglucoside, SB3-10 as detergents and TCEP (for DTT) as a reducing agent in addition to other components. Ionic detergents and materials containing sodium ions are not compatible with ProteomeLab PF2D chemistry. Ionic detergents apply a charge to the protein that is independent of the p*I*, which affects their elution. Sodium ions compete for the same position with acidic proteins on the chromatofocusing column, this causes them to elute prematurely. *n*-octylglucoside and SB3-10 are non-ionic and zwitterionic detergents which replaced SDS. Before commencement of chromatofocusing, careful calibration of the online pH monitor, pH adjustment of start and eluent buffers as well as pre-equilibration of the chromatofocusing column with start buffer were all conducted.

4.2.2.1 First dimension separation

Following protein extraction, an equivalent of 5 mg of crude protein was injected into the ProteomeLab PF2D system for first dimension separation (chromatofocusing). In chromatofocusing, proteins are separated based on their isoelectric point using an HPCF-1D column. Proteins with p*I* values above 8.5 were eluted from the chromatofocusing column in the first 90 min. The pH gradient commenced after 90 min and continued to 155 min. Removal of acidic proteins from the column by the NaCl wash and the final wash with water took 65 minutes. In total, the first dimension separation took approximately 220 min and resolved total proteins into 36 1^{st} dimension fractions. An example of the first dimension separation is illustrated in Fig. 4.3 which shows both the 1^{st} dimension chromatograms and pH profiles for the mock-treated and infected samples harvested after 6 hrs.

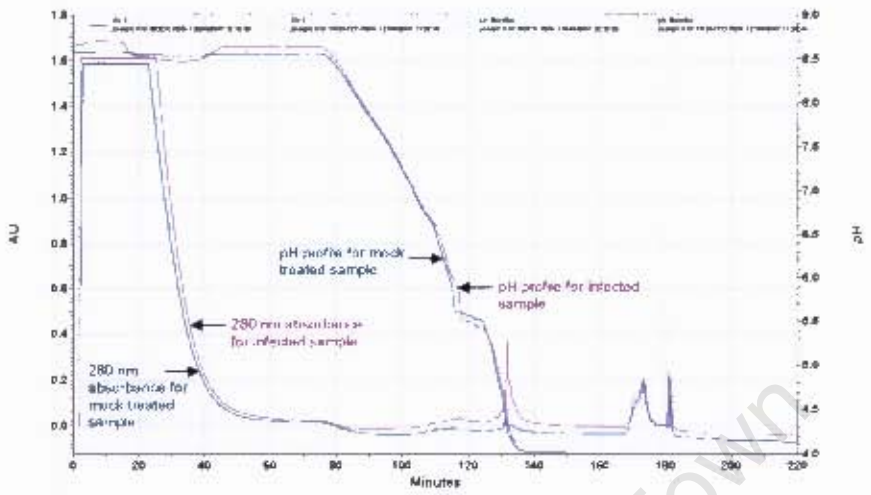


Figure 4.3: The 280 nm absorbance and pH profiles for the 1st dimension of *Arabidopsis* mock treated (blue) and infected (purple) leaf samples after 6 hrs. The left and right y-axis represent absorbance at 280 nm and pH respectively while the x-axis is time of the run in minutes. The column was first equilibrated with start buffer hence the peak that elutes before 60 minutes has a pI range greater than pH 8.5. When the 280 nm absorbance baseline is achieved, eluent buffer begins, and the pH gradient occurs between 80 and 130 min. When the pH gradient finishes, the column is washed with 1 M NaCl to remove the material that remains after pH 4.0 (after 130 min). The whole first dimension run took 220 minutes. This image also illustrates that 1st dimension separations of ProteomeLab PF2D are highly reproducible.

4.2.2.2 Second dimension separation

Second dimension separation was conducted using HPRP-2D column which was pre-equilibrated with 0.1% (v/v) TFA in water and its temperature maintained at 50°C. As already mentioned, proteins are separated based on their hydrophobicity in the second dimension. Each of the fractions resolved in the 1st dimension was further resolved into 54 fractions in the 2nd dimension. To determine which of the 54 fractions could be considered for mass spectrometry identification of proteins, each of the 36 fractions were pre-analyzed in a mapping mode. In this mode, 200 μL of each of the first dimension fractions was injected on to the second-dimension column and subsequently resolved to obtain chromatographic intensities, however, no second dimension fractions were collected at this stage. The resulting chromatographic intensities were analyzed using ProteoVue and DeltaVue as explained in section 4.2.2.3.

After determining first dimension fractions whose second dimension fractions displayed changes in absorbance, 500 μL of each of the selected first-dimension fractions was re-run and fractions collected in a 96-well plate by an automated fraction collector. In addition to the second dimension separation of each of the fractions collected before (basic), during and after (acidic) the pH gradient, basic and acidic fractions for each time point were combined to determine if significant changes can be observed. Figure 4.4 illustrates second dimension separation of combined basic fractions of treated samples, B4 (6 hrs), B8 (12 hrs) and B12 (24 hrs) and indeed changes can be observed across time points. One of the advantages of using ProteomeLab PF2D is the reproducibility of the resolutions which can be observed in the first (Fig. 4.3) and second (Fig. 4.4) dimension separations.

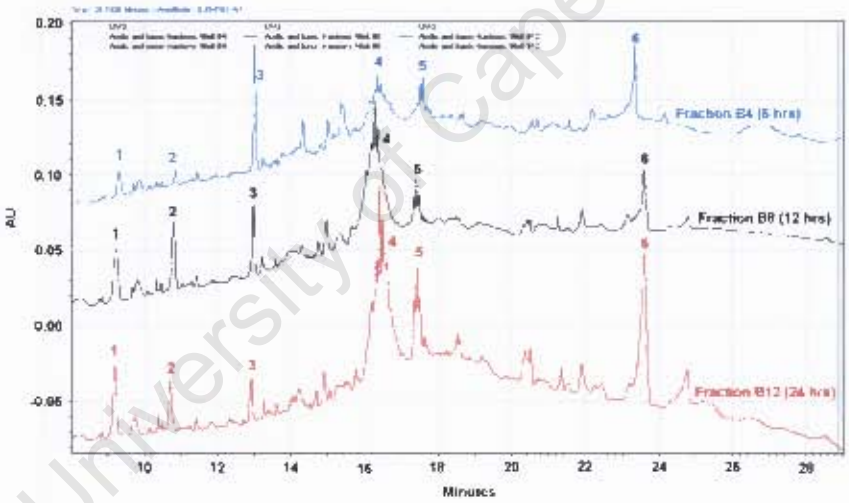


Figure 4.4: Comparison of 2nd dimension 214 nm profiles for *Arabidopsis* leaf tissue infected and harvested after 24 hrs for fractions B4 (6 hrs), B8 (12 hrs) and B12 (24 hrs). The three traces have been staggered to make comparison easier. The numbered peaks represent changes across time points. The image demonstrates the reproducibility of second dimension separations by ProteomeLab PF2D.

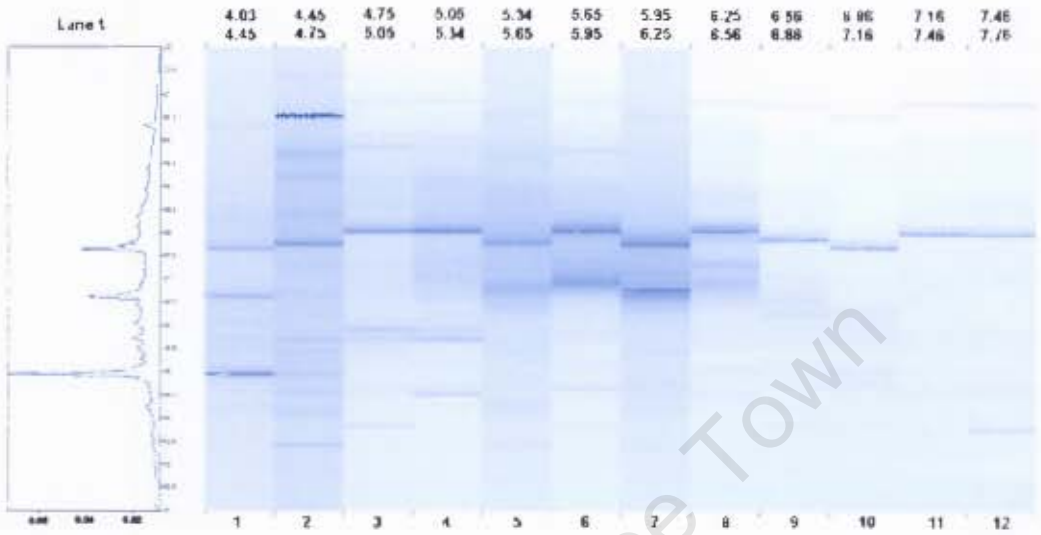
4.2.2.3 Selection of fractions for mass spectrometry

Chromatographic absorbance intensities from each p*I* fraction for one treatment (e.g. 6 hr mock treated sample) obtained from the second dimension fractionation were imported into ProteoVue software. In ProteoVue, these chromatographic intensities

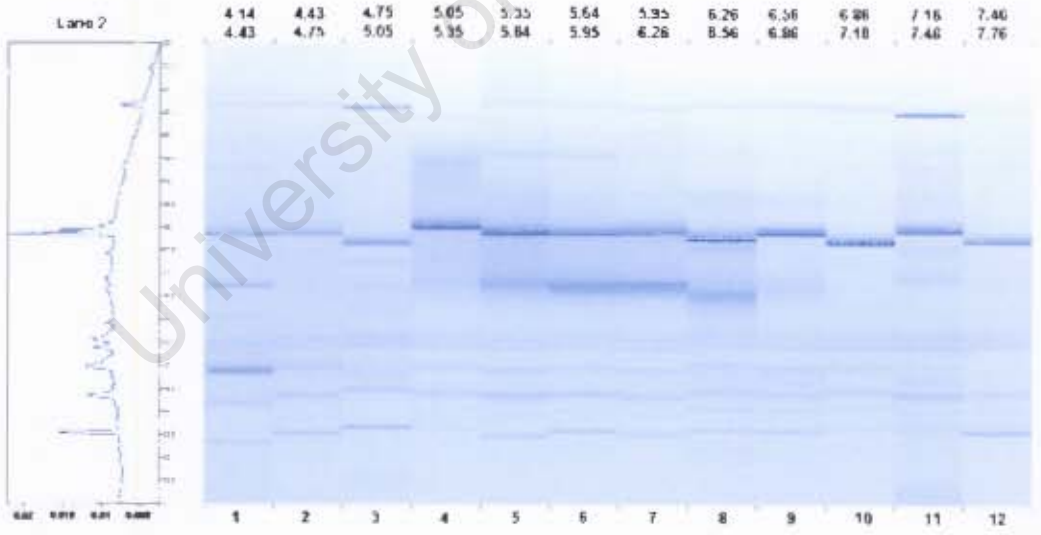
form a 2D LC map where the pI fractions are shown in the horizontal lanes and the UV chromatograms obtained in the second dimension are shown in the vertical position (Fig. 4.5). The map can be viewed in several coloured formats where the colour intensity is proportional to the relative intensity of each peak. The outputs of ProteoVue were imported into DeltaVue for differential analysis of corresponding fractions from two samples (e.g. 6 hpi mock vs 6 hpi samples) (Fig. 4.6).

DeltaVue provides semi-quantitative as well as quantitative information on expression level differences between two samples (mock and treated) that are being compared. This is achieved by comparing chromatogram peaks corresponding to the same proteins in the two samples allowing quantification by subtraction analysis. Expression level difference analysis was enabled by the pick peak function. Using this function, peaks that displayed differential expression between the mock and treated samples were selected. Fine adjustments were made on the intensities of these peaks using the baseline correction function. The essence of this action was to develop a more accurate expression level difference between the selected peaks.

Fractions containing peaks that displayed differential expression of more than 2 fold increase or reduction in absorbance between the mock and treated fractions were selected for mass spectrometry. Each of the selected fractions will contain several proteins. Because these fractions were selected based on absorbance change of the whole fraction, it is not known how many of the proteins are changing in absorbance, which of the proteins are changing and even if changes in one protein are masking changes (e.g. down-regulation) of another protein. Information on peaks that were selected is presented in Table. 4.4. Only a few peaks are presented for illustration. Figure 4.6A shows five peaks (1-5), information on these peaks has been presented in Table 4.4 under the 6 hr time point. The infections and protein extraction were replicated 3 times however, because of the immense number of chromatographic steps involved, only results for the first replicate have been obtained hence the last two replicates need to be analyzed to test the significance of the changes in protein absorbance. Although a number of proteins were fractionated by the system, only proteins contained in fractions that displayed differential expression were considered for mass spectrometry.

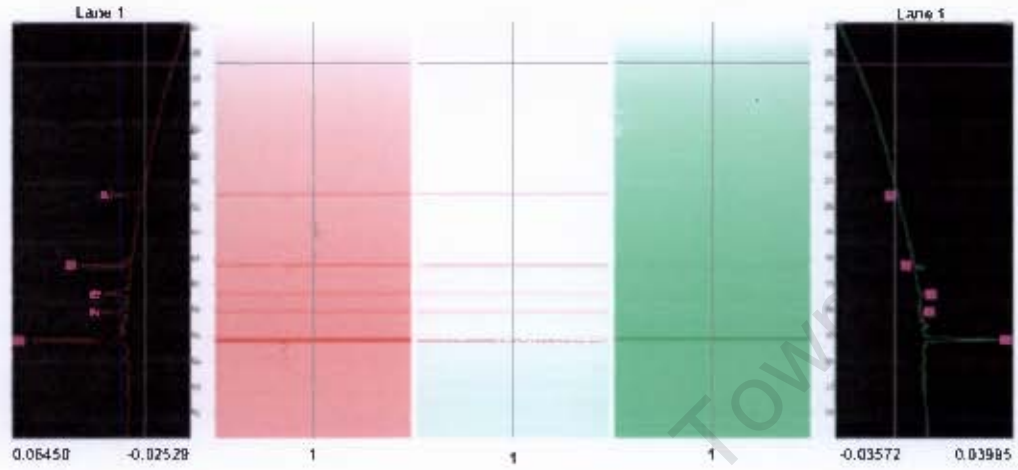


A

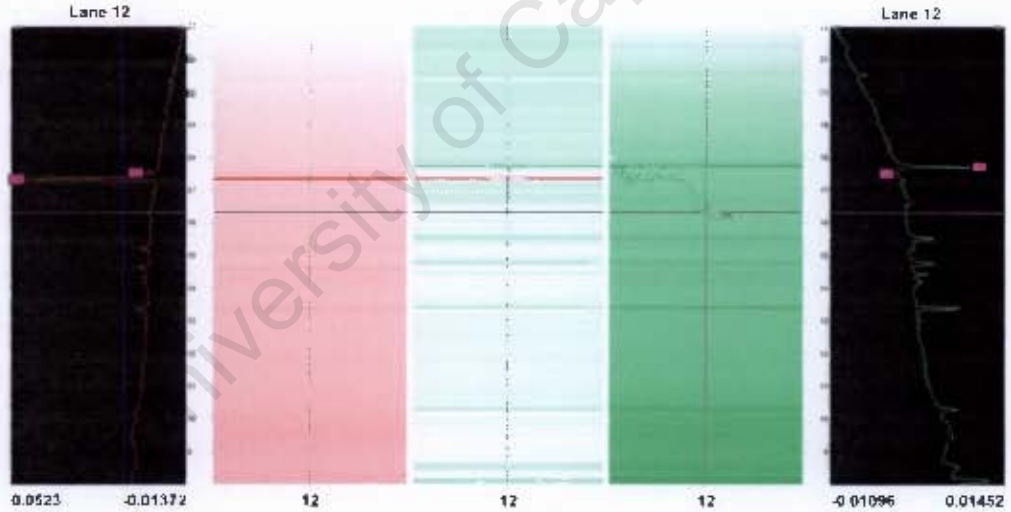


B

Figure 4.5: The two-dimensional map of infected *Arabidopsis* leaf tissue as viewed with ProteoVue. Each lane represents the absorbance intensity of the 2nd dimension separation of each fraction collected in the 1st dimension. At the top of each lane is the starting and ending pH of each 1st dimension fraction. To the left is the chromatogram for a given fraction, which is located with the cursor in the software. A common band which is possibly RuBisCO was fractionated after around 18 min in all fractions in the two samples, **A** (Infected 12 hpi replicate 1) and **B** (Infected 24 hpi replicate 1)



A



B

Figure 4.6: DeltaVue comparison of the two dimension protein map of *Arabidopsis* leaf tissue infected with *B. cinerea* (left, red) and mock treated leaf tissue (right, green). Each lane (e.g lane 4 shown above) represents the absorbance of the protein fraction while the UV chromatograms for the 2^{nd} dimension separation for corresponding lanes are shown to the outside of the map. The centre portion between the maps shows the qualitative and quantitative differences between samples where the intensity of the colour represents the abundance of the proteins associated with the peaks. Therefore the colour that is displayed corresponds to the sample with that protein in a greater amount. In **A** the infected sample has more protein than the mock treated sample while in **B** the mock treated has more protein than infected sample. Also in **B**, one peak is higher in mock and one peak is higher in the infected.

Table 4.4: Peak information of fractions that displayed more than 2 fold change in protein absorbance between samples mock-treated or infected and harvested after 6 hpi. Columns represent the peak number, lane, starting p*I*, ending p*I*, right retention time (RT), baseline correction for peaks in the right chromatogram (BR), ratio of peak area in the right and left chromatogram, baseline correction for peaks in the left chromatogram (BL) and right retention time (RT). The left and right chromatograms represent the infected and mock treated samples respectively.

Peak	Lane	Min p*I*	Max p*I*	RT	BR	L/R	R/L	BL	RT
6 hpi									
1	1	4.26	4.56	14.81	0.01	1.57	0.64	0.00	14.95
2	1	4.26	4.56	15.90	0.01	22.00	0.05	0.00	16.03
3	1	4.26	4.56	16.63	0.01	10.96	0.09	0.00	16.72
4	1	4.26	4.56	17.72	0.00	43.39	0.02	0.00	17.83
5	1	4.26	4.56	20.46	0.00	15.86	0.06	-0.01	20.56
6	2	4.56	4.86	17.71	0.01	39.72	0.03	0.00	17.82
7	3	4.86	5.17	20.44	0.00	35.53	0.03	-0.01	20.61
9	5	5.47	5.76	17.73	0.02	34.75	0.03	0.00	17.96
10	6	5.76	6.07	17.69	0.02	93.47	0.01	0.01	17.61
11	7	6.07	6.37	13.09	0.01	137.36	0.01	0.01	13.06
12 hpi									
2	1	4.26	4.56	15.90	0.01	22.00	0.05	0.00	16.03
3	1	4.26	4.56	16.63	0.01	10.96	0.09	0.00	16.72
4	1	4.26	4.56	17.72	0.00	43.39	0.02	0.00	17.83
5	1	4.26	4.56	20.46	0.00	15.86	0.06	0.01	20.56
7	2	4.56	4.86	14.84	0.01	5.38	0.19	0.00	15.00
8	2	4.56	4.86	17.71	0.01	39.72	0.03	0.00	17.82
9	2	4.56	4.86	20.47	0.00	16.72	0.06	0.01	20.59
10	3	4.86	5.17	17.70	0.00	4.45	0.23	0.01	17.82
11	3	4.86	5.17	20.44	0.00	35.53	0.03	0.01	20.61
24 hpi									
2	1	4.03	4.45	14.96	0.01	10.18	0.10	0.01	14.96
3	1	4.03	4.45	16.63	0.02	5.05	0.20	0.01	16.51
4	1	4.03	4.45	16.63	0.02	4.69	0.21	0.01	16.65
5	1	4.03	4.45	17.64	0.00	7.15	0.14	0.00	17.57
7	2	4.45	4.75	17.75	0.03	5.19	0.19	0.01	17.64
8	2	4.45	4.75	20.46	0.01	3.19	0.31	0.00	20.39
9	2	4.45	4.75	20.51	0.02	14.25	0.07	0.00	20.38
10	3	4.75	5.05	18.11	0.02	3.87	0.26	0.02	17.71
11	4	5.05	5.34	18.04	0.02	3.22	0.31	0.02	17.49
12	4	5.05	5.34	18.05	0.02	2.52	0.40	0.02	17.51
13	5	5.34	5.65	17.76	0.03	5.47	0.18	0.01	17.74
14	5	5.34	5.65	17.77	0.03	3.94	0.25	0.01	17.66

4.2.2.4 Proteins identified by mass spectrometry

In-solution digestion with trypsin was carried out on 175 μL of each of the selected second dimension fractions which had been dried under vacuum and rehydrated in ammonium bicarbonate buffer. The tryptic peptides were analyzed with an in-line liquid chromatography and electrospray ionisation mass spectrometry (LC-ESI-MS) using a Micromass Global Ultima mass spectrometer (Waters MS Technologies, UK). The instrument was operated in data dependent acquisition (DDA) mode. During the DDA analysis, both MS and MS/MS spectra was performed on the most intense peptides as they eluted from the chromatographic column. Processing of the spectra was performed using the Micromass ProteinLynx Global Server software package (Waters MS Technologies, UK), which then searched the MS/MS spectra against the EBI *Arabidopsis* protein database, release version 3.3 (http://www.ebi.ac.uk/IPI/IPIhelp.html) using the Micromass Global Server 2.2 search engine.

From the fractions that displayed an increase or a decrease in absorbance in infected compared to mock treated samples, protein identifications were made from all fractions selected at the three time points (6, 12 and 24 hrs). With the fractions selected to have displayed an increase in absorbance of proteins at 6 hrs after infection, only RuBisCO activase (At2g39730), a protein known to be down-regulated but highly abundant was identified and no other up-regulated proteins. The identification of only this protein suggests that either the mass spectrometry identification was not efficient or the concentration of up-regulated proteins at this time point was very low. Analysis of more replicates will help in ascertaining either of these hypotheses. Thirteen proteins were identified in fractions that displayed a decrease in absorbance. Examples of these proteins included RuBisCO activase (At2g39730), a protein kinase (At2g45590), Glutamine synthetase (At5g35630), Methionine Adenosyltransferase 3 (At2g36880) (Table 4.6.)

Although the number of selected fractions for 12 and 24 hr samples that demonstrated an increase in absorbance of more than 2 fold was more than those that demonstrated a reduction in absorbance, the number of up-regulated proteins identified in up-regulated fractions was far less than the number of down-regulated proteins identified in down-regulated fractions. One of the reasons could have been

the masking effect of high abundant proteins such as RuBisCO (At5g38430) and RuBisCO activase (At2g39730) as these proteins were identified almost in each and every fraction selected as up-regulated. This can be observed in Fig. 4.5 in which a protein most likely RuBisCO was eluted in each fraction between 17 and 18 min. The abundance of this protein probably hides a lot of less abundant protein changes in many fractions.

The number of proteins identified at 12 hrs as up-regulated was 5. With the exception of ascorbate peroxidase (At1g07890) the other 4 proteins (GSTs (At1g02920, At2g02930 and At4g02520) and catalase 3 (At1g20620)) (Table. 4.5) corresponded to genes up-regulated after 12 hrs in the microarray experiment. The number of proteins identified in fractions that displayed a reduction in absorbance were 73 (Table. 4.6). Like the 12 hr time point, only 8 proteins were identified in fractions selected as having increased in absorbance at 24 hrs and all the identified proteins corresponded to 8 genes up-regulated after 24 hrs in the microarray experiment. These proteins were a hevein-like protein (At3g04720), GSTs (At1g02920, At1g02930, At2g02930, At4g02520 and At5g40370), glutaredoxin (At5g40370), legume lectin family protein (At3g15356) and peroxidase (At3g49120) (Table. 4.5). Three of the proteins identified at 12 hrs were also present in infected leaves at 24 hrs (Fig. 4.7A). The number of proteins identified in fractions that displayed a reduction in absorbance were 55 (Table. 4.6). Two proteins were fractionated in all the three time points (Fig. 4.7B).

The ascorbate peroxidase protein identified in up-regulated fractions after 12 hrs also significantly increased in abundance after 24 hrs in the 2D SDS-PAGE experiment. The location of its spot (SSP 7101, 24 hrs infected) (Fig. 4.1) was correlated with gels at WORLD-2DPAGE. The gene corresponding to this protein was not significantly up-regulated after either time points in the microarray experiments. It is possible that the change in absorbance in that fraction was due to a different protein or the protein is post-transcriptionally regulated. One of the aims of comparing the transcriptome and proteome is to find such changes that would not be picked up in gene expression profiling studies such as microarrays. This experiment was conducted for only one replicate; therefore all the identified proteins have been included in tables 4.5 and 4.6. Most of these proteins were matched with

very few peptides which can be reflected in the very low percentage coverages hence they may not be believed as genuinely changing in abundance. However, because the genes encoding them were significantly up- or down-regulated in the microarray experiments strengthens the case that they may be indeed undergoing alteration.

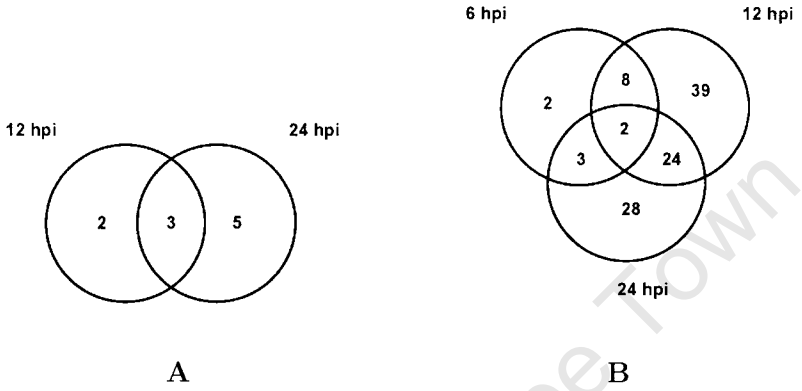


Figure 4.7: Four week old *Arabidopsis* leaf tissue was infected with *B. cinerea*. Proteins were extracted and fractionated by the ProteomeLab PF2D system. Shown are the number of proteins identified by mass spectrometry from fractions that displayed an increase in absorbance of more than 2 fold.

Table 4.5: Proteins up-regulated after 12 and 24 hpi in *B. cinerea* infected *Arabidopsis* leaf tissue compared to mock separated by 2D liquid chromatography using the ProteomeLab PF2D system. Columns report accession number (EBI database), gene locus, theoretical p*I* and MW, percentage coverage and protein description. The molecular weight (MW) and isoelectric point (p*I*) were calculated from the sequence of the protein in the database. The percentage coverage represents the amount of protein sequence covered by the matched peptides.

Accession	Locus	p*I*	MW	Coverage	Description
12 hrs					
IPI00548409	At1g02930	5.8	23471	4.8	Glutathione S-transferase 1
IPI00544607	At1g20620	6.3	48867	5.9	Catalase 3
IPI00532945	At2g02930	6.5	24106	3.8	Glutathione S-transferase 16
IPI00535149	At4g02520	5.9	24114	14.6	Glutathione S-transferase PM24
IPI00656658	At1g07890	5.9	27502	18.5	Ascorbate peroxidase 1
24 hrs					
IPI00607519	At1g02920	6.1	23583	11.0	Glutathione S-transferase 11
IPI00548409	At1g02930	5.8	23471	11.1	Glutathione S-transferase 1
IPI00532945	At2g02930	6.5	24106	13.2	Glutathione S-transferase 16
IPI00529373	At3g04720	7.9	22921	3.8	Hevein-like protein
IPI00535348	At3g15356	9.5	29593	4.0	Lectin like protein
IPI00522050	At3g49120	7.6	38807	7.1	Peroxidase 34
IPI00535149	At4g02520	5.9	24114	22.2	Glutathione S-transferase PM24
IPI00517541	At5g40370	6.7	11748	12.6	Glutaredoxin-C2

Table 4.6: Proteins down-regulated after 12 and 24 hpi in *B. cinerea* infected *Arabidopsis* leaf tissue compared to mock separated by 2D liquid chromatography using the ProteomeLab PF2D system. Columns report accession number (EBI database), gene locus, theoretical p*I* and MW, percentage coverage and protein description. The molecular weight (MW) and isoelectric point (p*I*) were calculated from the sequence of the protein in the database. The percentage coverage represents the amount of protein sequence covered by the matched peptides.

Accession	Locus	p*I*	MW	Coverage	Description
6 hrs					
IPI00520638	At1g32470	5.1	17886	9.6	Glycine cleavage system H protein 2
IPI00521992	At1g36940	10.3	20242	3.9	Unknown protein
IPI00541680	At1g42970	6.3	47630	2.2	GAPB
IPI00541637	At1g55040	6.6	94805	1.3	Zinc finger family protein
IPI00534914	At1g79330	6.2	44818	2.9	AMC6/ATMCP2B
IPI00527972	At2g36880	5.8	42471	3.9	Methionine Adenosyltransferase 3
IPI00520309	At2g39730	7.6	48469	22.9	RUBISCO activase
IPI00544162	At2g45590	8.8	75506	2.8	Protein Kinase famlity protein
IPI00522872	At3g26740	4.6	15304	12.1	CCL
IPI00519769	At3g50820	5.9	34998	18.7	Oxygen-evolving enhancer protein 1-2
IPI00534852	At5g35630	6.4	47381	1.9	Glutamine synthetase
IPI00656706	At5g38410	8.2	19383	4.6	RuBisCO small subunit 3B
IPI00545883	At5g66570	5.6	35120	33.7	Oxygen-evolving enhancer protein 1-1
12 hrs					
IPI00537832	At1g03130	9.8	22293	4.9	Photosystem I reaction center
IPI00531916	At1g03600	9.9	18823	5.7	Photosystem II family protein
IPI00535457	At1g03680	9.1	19652	14.0	Thioredoxin M-type 1
IPI00518864	At1g04410	6.1	35548	9.3	Malate dehydrogenase
IPI00540742	At1g06680	5.9	28078	3.8	Oxygen-evolving enhancer protein 1-2
IPI00846137	At1g07930	9.3	41348	3.0	Elongation factor 1-alpha
IPI00538278	At1g11860	8.6	44416	3.9	Aminomethyltransferase
IPI00846619	At1g12900	6.2	34311	7.6	GAPA-2
IPI00846497	At1g13440	6.8	33885	14.2	GAPC-2
IPI00524841	At1g13930	4.8	16154	16.1	Nodulin-related
IPI00846186	At1g19570	5.6	23440	3.8	Dehydroascorbate reductase
IPI00520177	At1g20340	5.1	16973	14.4	Plastocyanin major isoform
IPI00846719	At1g26630	5.7	15093	7.2	Eukaryotic translation initiation factor
IPI00525222	At1g30380	10.5	13198	6.9	Photosystem I reaction center
IPI00535877	At1g31330	9.6	24158	5.0	Photosystem I subunit F
IPI00520638	At1g32470	5.1	17885	9.6	Glycine cleavage system H protein 2
IPI00518517	At1g45249	9.4	43101	2.5	ABA responsive elements-binding factor
IPI00539020	At1g67090	7.6	20203	46.7	RuBisCO small subunit 1A
IPI00548733	At1g79850	10.6	16272	6.7	30s Ribosomal protein S17
IPI00520709	At1g80240	8.5	40200	2.2	Unknown protein

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Accession	Locus	p*I*	MW	Coverage	Description
IPI00533812	At2g03440	7.5	19689	5.9	Nodulin-related
IPI00657461	At2g21330	6.3	33302	7.4	Fructose-bisphosphate aldolase
IPI00529898	At2g21870	9.0	25144	5.0	ATP Synthase 24 KDa Subunit
IPI00528969	At2g24260	6.4	36499	2.6	Basic helix-loop-helix family protein
IPI00536157	At2g35370	5.2	17935	9.7	Glycine cleavage system H protein 1
IPI00526310	At2g36530	5.5	47689	4.7	Enolase
IPI00527972	At2g36880	5.8	42470	3.8	Methionine adenosyltransferase 3
IPI00540246	At2g37220	5.1	30699	3.1	Putative ribonucleoprotein
IPI00527785	At2g38540	9.3	11747	15.3	Non-specific lipid-transfer protein 1
IPI00520309	At2g39730	7.6	48469	15.4	RuBisCO activase (RCA)
IPI00519410	At3g13470	5.6	63302	6.0	Chaperonin
IPI00846962	At3g15020	9.5	33113	5.1	Malate dehydrogenase
IPI00529886	At3g15360	9.6	21159	10.9	Thioredoxin M-type 4,
IPI00536966	At3g17390	5.5	42768	4.8	S-adenosylmethionine synthase 3
IPI00518644	At3g22890	6.3	51427	2.2	ATP Sulfurylase 3
IPI00537303	At3g26650	7.6	42463	2.0	GAPA
IPI00519769	At3g50820	5.9	34998	25.1	Oxygen-evolving enhancer protein 1-2
IPI00523226	At3g52150	6.8	59745	2.0	Uncharacterized protein
IPI00533612	At3g52960	9.1	24669	4.7	Peroxiredoxin-2E
IPI00525581	At3g60210	7.7	15131	6.5	Chloroplast chaperonin 10
IPI00517879	At3g62030	8.8	28190	3.5	Peptidyl-prolyl cis-trans isomerase
IPI00535044	At3g62410	4.8	14157	9.9	CO12-2
IPI00516646	At3g63140	8.5	43903	2.5	Uncharacterized protein
IPI00545948	At3g63190	9.5	30403	3.6	Ribosome recycling factor
IPI00521950	At4g01900	9.2	21262	5.6	Glutamine synthetase B1
IPI00530995	At4g03280	8.6	22518	6.7	Isoform 2 of Cytochrome B6-F complex
IPI00525302	At4g04640	8.1	40886	3.5	ATP synthase gamma chain 1
IPI00548616	At4g05180	9.7	24628	19.1	Oxygen-evolving enhancer protein 3-2
IPI00656759	At4g08870	6.6	29295	3.8	Arginase
IPI00521214	At4g09320	8.4	18802	5.3	Nucleoside diphosphate kinase 1
IPI00535216	At4g10340	6	30138	3.6	Chlorophyll A-B binding protein CP26
IPI00530817	At4g18480	6.1	46241	2.4	Magnesium-chelatase subunit CHLI
IPI00532377	At4g20260	5	24568	5.8	DREPP plasma membrane polypeptide
IPI00532582	At4g21280	9.6	23781	9.9	Oxygen-evolving enhancer protein 3-1
IPI00531287	At4g24280	5.1	76461	1.3	Chloroplast heat shock protein 70-1
IPI00546869	At4g27520	9.4	35042	2.6	Early nodulin-like protein 2
IPI00532440	At4g28750	9.9	14958	9.1	Photosystem I reaction center subunit IV
IPI00525727	At4g37930	8.1	57364	8.9	Serine hydroxymethyltransferase
IPI00541448	At4g38970	6.8	42915	8.0	Fructose-bisphosphate Aldolase 2
IPI00519631	At5g03850	10.8	7366	18.8	40S ribosomal protein S28-1
IPI00657400	At5g09660	7.6	34953	3.3	Peroxisomal NAD-malate dehydrogenase
IPI00523587	At5g14740	5.4	28326	6.6	Carbonic anhydrase 2

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Accession	Locus	p*I*	MW	Coverage	Description
IPI00537160	At5g15970	9.1	6547	37.9	Stress-induced protein kin2
IPI00521944	At5g26780	8.8	57305	1.7	Serine hydroxymethyltransferase 2
IPI00534852	At5g35630	6.4	47381	7.4	Glutamine synthetase
IPI00656706	At5g38410	8.2	19383	16.7	RuBisCO small subunit 3B
IPI00523477	At5g38420	7.6	20337	33.7	RuBisCO small subunit 2B
IPI00521186	At5g38430	7.8	20273	24.9	RuBisCO small subunit 1B
IPI00518961	At5g49910	5.2	76949	1.8	Heat shock protein 70-7
IPI00523656	At5g55220	5.3	61695	2.4	Trigger factor type chaperone
IPI00531316	At5g63400	6.9	26915	6.1	Adenylate kinase 1
IPI00547610	At5g64040	9.1	18417	29.8	Photosystem I reaction center subunit N
24 hrs					
IPI00531916	At1g03600	9.9	18823	7.5	Photosystem II family protein
IPI00518864	At1g04410	6.1	35548	2.4	Malate dehydrogenase
IPI00846137	At1g07930	9.3	41347	3.0	Elongation factor 1-alpha
IPI00846497	At1g13440	6.8	33884	4.5	GAPC-2
IPI00524194	At1g15820	6.8	27505	3.9	Light harvesting complex PSII
IPI00520177	At1g20340	5.1	16973	14.4	Plastocynin major isoform
IPI00543566	At1g53240	8.5	35781	7.3	Malate dehydrogenase 1
IPI00533812	At2g03440	7.5	19688	5.9	Nodulin-related
IPI00541933	At2g21660	5.4	15539	13.2	Cold, circadian rhythm
IPI00525237	At2g28000	5.1	62033	3.4	Chaperonin-60alpha
IPI00527785	At2g38540	9.3	11746	8.5	Non-specific lipid-transfer protein 1
IPI00520309	At2g39730	7.6	48469	2.5	RuBisCO activase (RCA)
IPI00544162	At2g45590	8.8	75506	2.8	Protein Kinase famlity protein
IPI00527415	At3g01390	5.8	12389	20.9	Vacuolar ATP synthase subunit G 1
IPI00657469	At3g01500	5.3	28180	18.9	Carbonic anhydrase 1
IPI00521134	At3g06050	9.0	21432	5.5	Peroxiredoxin-2F
IPI00846962	At3g15020	9.5	33112	3.8	Malate dehydrogenase
IPI00522229	At3g16140	10.0	15207	7.6	Photosystem I reaction center
IPI00529853	At3g20390	9.2	27782	23.9	Translational inhibitor protein
IPI00548978	At3g26060	9.5	23663	7.4	Peroxiredoxin Q
IPI00525750	At3g47070	9.7	10523	26.0	Thylakoid soluble phosphoprotein
IPI00519769	At3g50820	5.9	34997	3.6	Oxygen-evolving enhancer protein 1-2
IPI00523226	At3g52150	6.8	59744	2.0	Uncharacterized protein F4F15.260
IPI00532442	At3g53430	9.1	17958	9.0	60S ribosomal protein L12-2
IPI00535044	At3g62410	4.8	14157	9.9	CP12-2
IPI00545948	At3g63190	9.5	30403	3.6	Ribosome recycling factor
IPI00529234	At4g01150	9.2	17686	6.1	Uncharacterized protein
IPI00530995	At4g03280	8.6	22518	5.7	Isoform 2 of Cytochrome B6-F complex
IPI00521214	At4g09320	8.4	18801	5.3	Nucleoside diphosphate kinase 1
IPI00528276	At4g09650	9.1	25652	4.3	ATP synthase delta chain

Continued on Next Page...

Accession	Locus	p*I*	MW	Coverage	Description
IPI00535216	At4g10340	6.0	30137	3.6	Chlorophyll A-B binding protein CP26
IPI00533660	At4g10790	4.9	52770	2.1	UBX domain-containing protein
IPI00519731	At4g14880	7.0	52980	2.3	Cysteine synthase
IPI00846603	At4g18360	7.7	34382	3.2	(S)-2-hydroxy-acid oxidase
IPI00520474	At4g20360	5.8	51597	2.3	Elongation factor TU
IPI00532582	At4g21280	9.6	23780	30.5	Oxygen-evolving enhancer protein 3-1
IPI00544207	At4g21850	7.6	13512	7.4	Methionine sulfoxide reductase
IPI00531287	At4g24280	5.1	76461	3.1	Chloroplast heat shock protein 70-1
IPI00546869	At4g27520	9.4	35042	2.6	Early nodulin-like protein 2 precursor
IPI00532440	At4g28750	9.9	14957	9.1	Photosystem I reaction center subunit IV
IPI00534382	At4g34870	8.9	18366	8.1	Peptidyl-prolyl cis-trans isomerase
IPI00525727	At4g37930	8.1	57364	2.3	Serine hydroxymethyltransferase
IPI00516234	At5g08690	6.2	59676	4.1	ATP synthase subunit beta-2
IPI00522652	At5g13710	5.9	38244	2.4	Cycloartenol-C-24-methyltransferase
IPI00524759	At5g20630	6.3	21822	5.2	Germin-like protein
IPI00521186	At5g38430	7.8	20273	12.2	RuBisCO small subunit 1B
IPI00549113	At5g38570	6.8	47719	1.7	FBD-associated F-box protein
IPI00542973	At5g40770	7.0	30381	4.0	Prohibitin 3
IPI00532635	At5g45680	9.0	22025	8.7	FK506-binding protein 1
IPI00518961	At5g49910	5.2	76949	3.1	Heat shock protein 70-7
IPI00527963	At5g56210	4.9	56479	2.6	Unknown protein
IPI00519434	At5g57950	5.2	30114	2.9	Genomic DNA
IPI00542817	At5g59880	5.0	14115	9.7	Actin depolymerizing factor 3
IPI00657184	At5g63400	6.3	20805	7.4	Adenylate kinase 1
IPI00525776	Atcg00480	5.4	53900	11.0	ATP synthase subunit bate

4.2.2.5 Functional categorization

Proteins identified from up- and down-regulated fractions were subjected to functional categorization based on the biological process gene ontology at http://fatigo.bioinfo.cipf.es to look for over-represented (proportion (%)) functional categories (Ashburner *et al.*, 2000). Functional categories at the gene ontology level 3 whose P values (cut-off of $P \leq 0.01$) had been adjusted for multiple testing were considered significant. The functional categories over-represented for proteins up-regulated after 12 hrs were response to chemical stimulus and catabolic process while secondary metabolic process was over-represented for proteins up-regulated after 24 hrs (Table 4.7). Photosynthesis, carbon utilization, response to abiotic stimulus and

nitrogen compound metabolic process functional categories were over-represented for proteins down-regulated after 12 while photosynthesis and carbon utilization were over-represented in protein up-regulated after 24 hrs (Table 4.7). Even though there was a greater over-representation of responsive genes in terms of proportion, the functional categories of response to stress and secondary metabolic process for proteins up-regulated after 6 hrs; photosynthesis and carbon utilization for proteins down-regulated after 6 hrs as well as response to chemical stimulus and catabolic process for proteins up-regulated after 24 hrs were not considered significant since their P values were greater than the cut-off (data not shown). Results obtained in this analysis support those obtained with the analysis of up- and down-regulated genes in the two microarray experiments.

Table 4.7: Functional categorization of up- and down-regulated proteins in the 2D liquid chromatography experiment based on biological process. Only over-represented functional categories are shown.

	Responsive genes		**Whole genome**		
Entity	Number	Percentage	Number	Percentage	P-value
Up-regulated proteins					
12 hpi					
Response to chemical stimulus	5	100	939	8.4	1.72 E-03
Catabolic process	4	80	473	4.2	4.40 E-03
24 hpi					
Secondary metabolic process	5	57.1	280	2.5	5.46 E-03
Down-regulated proteins					
6 hpi					
12 hpi					
Photosynthesis	3	30	76	0.7	3.43 E-10
Carbon utilization	7	20	13	0.1	4.06 E-10
Response to abiotic stimulus	14	24.1	651	5.8	6.13 E-04
Nitrogen compound metabolic process	10	17.2	325	2.9	8.11 E-04
24 hpi					
Photosynthesis	11	8.4	43	0.4	3.38 E-27
Carbon utilization	12	2.8	3	0.0	3.21 E-12

These results have demonstrated that the role of antioxidants seems to be significant in this interaction. Some of the proteins look like they are up-regulated at transcriptional level while others may be regulated post-transcriptionally. Functional categorization has also demonstrated that the over-representation of the functional categories of secondary metabolic processes and photosynthesis can also be observed at the proteome level.

4.3 Discussion

The overall aim was to examine changes in protein abundance during infection with two aims i) determining if there is an increase or reduction in abundance of proteins corresponding to genes up- or down-regulated in the microarray study respectively; b) finding proteins whose levels may be post-transcriptionally regulated. To achieve these aims, two techniques, 2D SDS-PAGE and ProteomeLab PF2D were employed to study the defence proteome of *Arabidopsis* after infection with *B. cinerea*. Both techniques demonstrated that infection of *Arabidopsis* with *B. cinerea* resulted in significant changes in the defence proteome as early as 6 hrs. Although both these techniques were used, each of them has its own limitations. For instance, with 2D SDS-PAGE, the main limitations include irreproducibility, gel-to-gel variation as well as less sensitivity of the most affordable coomassie dye. Reproducibility may be obtained where gels are run concurrently like in this study.

With proteomeLab PF2D, selection of fractions for protein identification is based on absorbance, however, the proteins contained in each fraction are not known. While proteins are identified in the selected fractions, these fractions may still contain several other proteins hence can not determine which of them are changing between samples. Because fractions may contain many proteins, changes in low abundant proteins can be missed as they may be masked by changes in abundant proteins. Another limitation of ProteomeLab PF2D is the amount of chromatographic steps involved which translates into a lot of time needed to run the whole experiment. This is the sole reason why only one set of samples was run in the 2D LC experiment. For instance, the 1^{st} dimension fractionates total protein into 36 fractions while the 2^{nd} dimension fractionates each of the 1st dimension fractions into 54

fractions. This translates into 1,944 fractions for one treatment (e.g. mock treated sample) and 11,664 fractions for the whole replicate. Although only one sample can be run at a time, the runs are highly reproducible unlike in 2D SDS-PAGE.

In terms of amount of protein required, the 2D SDS-PAGE requires a lot less protein than ProteomeLab PF2D. For example, separation on mini gels (7 cm strip) requires about 200-500 μg with coomassie staining or 10-100 μg (7 cm) with silver or SYPRO Ruby staining while large gels (11 and 17 cm strips) require 200-1,000 μg and 1-3 mg with coomassie staining, 50-200 μg (11 cm), 100-300 μg (17 cm) with silver or SYPRO Ruby staining respectively (BioRad, Laboratories Inc, Hercules, USA). With ProteomeLab, 5 mg are required for injection for first dimension separation. Even when a RuBisCO depletion kit which has been described in section 4.3.5 is used, at least 1 mg of crude protein is required for each column.

4.3.1 Responses of *Arabidopsis* to *B. cinerea* occur in early time points

The 2D SDS-PAGE experiment demonstrated that 9 protein spots displayed an increase in abundance after infection by 6 hrs while 6 protein spots displayed reductions in abundance. The differential map of the 2D LC system ProteomeLab PF2D also demonstrated changes in protein abundance as early as 6 hrs. These results support earlier observations made in the microarray study in which *OXI1* expression, H_2O_2 and camalexin accumulation were all shown to occur in early time points. Put together, these results suggest that *Arabidopsis* responds to infection by *B. cinerea* very early in the interaction. Since the 2D SDS-PAGE experiment was highly reproducible, it is worth performing the same experiment but with early time points and with large gels. This should provide enough protein for mass spectrometry identification so as to determine the type of proteins changing in these early time points. Such proteins may be very essential in the interaction and provide good candidates for breeding programs to reduce susceptibility not only to *B. cinerea* but also other pathogens as observed in cluster analysis (Chapter 2).

4.3.2 GSTs may be targeting toxic compounds from *B. cinerea* in addition to AOS

The microarray experiment demonstrated the up-regulation of genes encoding proteins involved in the detoxification of AOS. In this study the abundance of these proteins was shown to increase in *Arabidopsis* tissues infected by *B. cinerea*. The fractionation of a catalase 3 which mediates the breakdown of H_2O_2 suggests the presence of AOS. The presence of AOS in *B. cinerea* interactions is known and has been demonstrated in many studies (Govrin and Levine, 2000; Muckenschnabel *et al.*, 2001; Schouten *et al.*, 2002; Muckenschnabel *et al.*, 2003). The interesting observation however, was the separation as well as fractionation of GSTs by 2D SDS-PAGE and ProteomeLab PF2D respectively. Besides their role in the detoxification of AOS, GSTs have been shown to be important in the biotransformation of many xenobiotic substances. It is known that *B. cinerea* produces a number of pathogenicity factors such as the nonhost specific toxins like botrydial (Deighton *et al.*, 2001; Colmenares *et al.*, 2002)and oxalic acid (Han *et al.*, 2007). Both these compounds have very potent effects and result in massive maceration of host cells. The over-representation of GSTs suggests that they may also be aimed at a number of these toxins produced by *B. cinerea*.

4.3.3 Lectins may play a significant role in resistance to *B. cinerea*

The lectins were significantly up-regulated in the microaarray experiments. The fact that a lectin was fractionated in this experiment demonstrates that they may play a significant role in host resistance to *B. cinerea*. Sharon and Lis (2004) recently demonstrated that a potato lectin inhibits sporulation and growth of *B. cinerea*. Lectins have also been shown to cause growth disruptions during germination of *N. crassa*, *Aspergillus amstelodami*, and *Botryodiplodia theobromae*. In addition to pathogens, lectins have also been shown to have activity over insects. For instance, feeding bruchid beetles with a diet containing the black bean lectin resulted in the death of the bruchid larvae. All these studies demonstrate that this type of proteins are very important in host resistance.

4.3.4 The down-regulation of the photosynthetic pathway is also observable at the proteome level

The microarray study demonstrated the down-regulation of genes encoding enzymes involved in the photosynthetic pathway. This effect was subsequently visualized using MapMan. Although protein spots that displayed a significant reduction in abundance in the infected compared to mock treated samples in the 2D SDS-PAGE experiment were not identified, one of the down-regulated spots from gels in this study was correlated with gels at WORLD-2DPAGE and found to be RuBisCO. A gene encoding this protein was shown in the microarray study to be down-regulated. RuBisCO together with other proteins involved in photosynthesis were also identified in down-regulated fractions following fractionation by the 2D LC system, ProteomeLab PF2D. Like in the microarray experiment, functional categorization of all proteins identified in down-regulated fractions demonstrated the over-representation of the functional category of photosynthesis.

4.3.5 ProteomeLab PF2D; the necessity for optimization in plant projects

Although the proteomeLab PF2D system has been shown to provide invaluable information regarding differential expression of proteins, the system did not identify as many changes in absorbance of fractions as expected and subsequently as many proteins in these fractions as expected. However, this system can still provide beneficial information in relation to *Arabidopsis* defensive responses after infection by *B. cinerea*. For the above to be realized, two things may need to be done. First, the protein extraction protocol needs to be optimized for particular plant samples. This assumption is supported by the study of Pirondini and associates (2006). They used two different protocols, the $MgSO_4$-based extraction protocol and urea-based extraction protocol (Beckman Coulter) also used in this study. They showed that a higher amounts of proteins were detected when the $MgSO_4$-based extraction protocol was used than the urea-based extraction protocol. This protocol was not adopted in this study partly because it would increase the cost of the project to test it through the whole proteomeLab PF2D system on our samples. However, these results have confirmed the necessity for performing this optimization and it is necessary in future

studies if the ProteomeLab PF2D system is to be used on plant samples.

Secondly, it is important that RuBisCO is removed from crude plant protein extracts before first dimension separation. It was observed in this study that RuBisCO was present in almost all of the fractions which could have masked the detection of proteins that change in abundance. GenWay (http://www.genwaybio.com), a corporate partner of Beckman Coulter, Inc. recently developed a kit (Seppro® RuBisCO) which specifically removes RuBisCO from crude plant protein extracts. This kit is composed of a column that is based on avian antibody (IgY)-antigen interactions and buffers optimized for sample loading, washing, elution and regeneration. RuBisCO is selectively removed by the immobilized specific IgY when crude biological samples are passed through the column. The kit can be used with either the 2D SDS-PAGE or 2D LC platform. It was not possible to use this kit because it was not yet available. The use of this kit will reduce the amount of protein needed to be injected into the system for first dimension separation. An equivalent of 5 mg of total protein was injected into the system. This high amount of protein resulted in clogging of columns especially the second dimension (HPRP) column which performs a number of resolutions. This resulted in high back pressure in the system in addition to affecting the stability of retention times. As a result, columns could not be used for so many runs without cleaning and in some cases they had to be completely replaced. Loading less protein will not only increase the life span of the columns especially the HPRP column but also significantly reduce the cost of ProteomeLab PF2D plant projects.

4.3.6 The iTRAQ reagent; another option for studying the *Arabidopsis* defence proteome

In addition to ProteomeLab PF2D, another technique that may be used to study the defence proteome of *Arabidopsis* following infection by *B. cinerea* is the iTRAQ reagent (Applied Biosystem). The iTRAQ reagent is an amine group based isotope labeling methodology. It consists of three moieties; a peptide group, a reporter group and a balance group of which the last two groups constitute an isobaric tag. The peptide group which is an NHS ester group covalently links the isobaric tag to peptides by reacting with free primary amines at the amino-termini and lysine

side chains. The reporter group is a tag with a mass of 114, 115, 116 or 117 Da, depending on differential isotopic combinations of $^{12}C/^{13}C$ and $^{16}O/^{18}O$ in each individual reagent. The balance group ranges in mass from 28 to 31 Da to ensure that the combined mass of the reporter and balance groups remains constant for all four reagents. Therefore, peptides labeled with different isotopes are isobaric and are chromatographically indistinguishable, a factor that is important for accurate quantitation. During collision-induced dissociation, the reporter group ions fragment from the backbone peptides, displaying distinct masses of 114 to 117 Da. The intensity of these fragments is used for quantitation of the individual representative peptides. Relative peptide abundance is then quantified from the MS/MS spectra. The iTRAQ reagent offers a more protein coverage and accurate quantification since it is based on detection of a reporter that fragments during MS/MS. In addition, up to four distinct samples can be analyzed in a single experiment and involves fewer chromatographic steps which reduces sample loss, cost of the project and saves time.

4.4 Conclusion

This study employed two techniques to study the defence proteome of *Arabidopsis* after infection with *B. cinerea* with the view of determining if there is any correlation between up- or down-regulation of genes and expression or repression of proteins respectively. This objective was met because the proteins identified after separation by 2D SDS-PAGE or fractionation by the 2D LC system ProteomeLab PF2D were those identified in the microarray experiments. Functional categorization also demonstrated the over-representation of the functional category of photosynthesis among down-regulated proteins as also observed with genes down-regulated in the microarray experiments which is another confirmation of microarray data. This study also demonstrated that changes in the proteome occur as early as 6 hrs following inoculation. This was in support of earlier results in which H_2O_2 was detected around inoculated sites of *Arabidopsis* leaves with no visible lesions by 6 hrs. Glutathione S-transferases were identified in proteins separated by 2D SDS-PAGE and fractionated by ProteomeLab PF2D. This result demonstrates that GSTs may be playing a significant role in this interaction possibly detoxifying toxic pathogenicity factors produced by *B. cinerea* in addition to neutralization of AOS.

The ProteomeLab PF2D system did not identify as many changes in absorbance of fractions as expected and subsequently as many proteins in these fractions as expected. It will be necessary to optimize protocols for extracting plant proteins for this system while the removal of RuBisCO will not only enhance resolution but will also increase sensitivity. However, the fact that in many cases the proteins identified in fractions with increase or decrease in absorbance did represent genes up- and down-regulated on the array suggests that this system can be used to identify proteins changing in abundance in plant host/pathogen interactions. The iTRAQ reagent may also provide another option to ProteomeLab PF2D. It can analyze up to four samples unlike the one sample for ProteomeLab PF2D and involves fewer chromatographic steps. One of the aims was also to identify relatively low abundance proteins especially in early time points which may have a role in signal transduction such as transcription factors and protein kinases however, it seems that only highly abundant proteins were identified. Low abundant proteins exist in very low concentrations. Washburn and associates (2002) reported that abundant proteins are identified with multiple peptides while low abundant proteins were identified with very few peptides either one or two. Two options may be employed to get around this problem which include depletion of RuBisCO and enrichment of low abundant proteins through protein prefractionation.

Chapter 5

General Discussion

5.1 Global food security at the mercy of plant diseases

The World's population is expected to grow to an unprecedented level of 8.1 billion by the year 2030 which is on average, a growth rate of around 750 million people per year (Pinstrup-Andersen *et al.*, 1999; FAO, 2000). To feed this many people, twice as much food as that produced today will be needed. Hence the reason of concern today is not the escalating world population but the slowly expanding global food supply (Dyson, 1999; Huang *et al.*, 2002). One of the main factors known to influence global food production are diseases of plants caused by a multitude of organisms which include viruses, bacteria and fungi (Strange and Scott, 2005). Global crop losses attributable to these yield decimating organisms annually have been estimated at 10% (Strange and Scott, 2005). A number of strategies to manage diseases caused by these organisms have been put in place over the years however, existing pathogens have become more fit while at the same time, new diseases are developing in areas where they have never been reported (Gouda and Emeran, 2007; Papayiannis *et al.*, 2007; Shih *et al.*, 2007; Zea-Bonilla *et al.*, 2007).

Successful development of epidemics is controlled by three factors; the environment which must be very favourable for the pathogen to flourish, the pathogen which must be very virulent and the host which must be susceptible. This makes up what is referred to as the disease triangle (Agrios, 1997). Therefore disease control strategies are aimed at exploiting any of the factors within the triangle so as to achieve successful disease management. For instance, cultural control strategies are aimed at creating a clean and safe environment for crop growth (Trolinger and Strider, 1984; Hausbeck and Moorman, 1996; Williamson *et al.*, 2007) while biological and chemical control strategies target the pathogen reducing its ability to cause disease (Masner *et al.*, 1994; Miura *et al.*, 1994; Elmer and Reglinski, 2006). Although these strategies may play a vital role in limiting epidemic development, the role the environment and the pathogen play in initiating and sustaining epidemics is in most cases beyond our ability to control. However, we can limit disease development by manipulating the third factor within the disease triangle, which is the host, by engineering it to become more resistant to pathogens (Campbell *et al.*, 2002). Through employing an integrated approach, we can then achieve successful

management of various diseases. Developing crop varieties that resist diseases and tolerate adverse climatic conditions such as drought is one of the overriding goals of most National Agricultural Research Systems (NARS) in sub-Saharan Africa and many other countries in the world (Komen and Persley, 1993).

5.2 Global transcriptome profiling in host-pathogen interactions

Developing crop varieties which can withstand adverse abiotic and biotic stresses requires an in-depth understanding of the molecular mechanisms employed by plants during such conditions. This information can be generated by studying expression profiles of genes in plants exposed to either abiotic or biotic stresses. Microarrays provide an opportunity for probing changes in gene expression profiles but on a global scale (Allemeersch *et al.*, 2005; Rensink and Buell, 2005). Use of a model system coupled with microarrays provides another opportunity for easy interpretation of the generated information (Chen *et al.*, 2004; Koide *et al.*, 2005). The results from such experiments can then be extrapolated to real crop situations. In this work we employed the model crucifer *Arabidopsis thaliana* and the necrotrophic deuteromycete *Botrytis cinerea* (Meinke *et al.*, 1998; Rensink and Buell, 2004; Tivoli *et al.*, 2006).

A. thaliana was well suited for this kind of experiment because of the large number of pathogens reported to infect it including *B. cinerea* (Buell and Somerville, 1997; Kunkel and Brooks, 2002). Because the *Arabidopsis* genome is sequenced (AGI, 2000), *Arabidopsis* microarray chips are also readily available from a number of platforms (Rensink and Buell, 2005). *B. cinerea* was chosen as the pathogen of choice because of its importance in the horticultural industry (Williamson *et al.*, 2007), one of the most growing agricultural sectors in the world (http://www.worldbank.org/agsourcebook). Equipped with information from global expression profiling, it is possible to identify candidate genes that can be strategically employed in biotechnology to enhance resistance in susceptible plants. This strategy has been employed before and shown to be effective. For instance, Chern and associates (2001), transformed the *Arabidopsis NPR1* gene into rice and demon-

strated that resistance of transgenic rice plants to the rice bacterial blight pathogen (*Xanthomonas oryzae* pv. *oryzae*) was enhanced.

Resistant and susceptible plants both contain the same type of genes however, the difference between the two is that resistant plants signal the presence of pathogens and respond swiftly to deter its continued development. Based on this, two microarray experiments were carried out to identify genes induced both in time and space as they may have a significant role in impeding colonization of the plant by the pathogen. Infection of *Arabidopsis* with *B. cinerea* resulted in the expression as well as repression of a number of genes both temporary and spatially. By using different gene expression profiling techniques, the expression profiles of these genes were found to be consistent and therefore reliable. A number of genes whose role in resistance against *B. cinerea* had been earlier elucidated such as patatin (*At2g26560* and *At4g37060*) (La Camera *et al.*, 2005), Bax inhibitor (*At5g47120*) (Sanchez *et al.*, 2000) were also identified in the arrays which is another form of validation.

A number of studies have shown that levels of expressed mRNAs may not always correlate with levels of expressed proteins (Gygi *et al.*, 1999; Ideker *et al.*, 2001; Kern *et al.*, 2003) yet proteins are the main working molecules in cells (Lodish *et al.*, 2004). Hence in addition to validation of microarray expression profiles, proteomic studies are very important especially in identifying proteins whose abundance increases at early time points as these may play significant roles in defence and hence very essential in breeding programs. However, it is necessary first to identify such proteins especially in a modal pathosystem such as that involving *Arabidopsis* and then demonstrate that they have a role in defence. These can then be identified in crop plants followed by demonstration of their role in defence in those plants. The inclusion of proteomic studies in identification of genes necessary for enhancing resistance in susceptible plants is supported by this and many other studies which have shown that there may not always be a correlation between high levels of expression of genes and resistance. Infection of *Arabidopsis* with *B. cinerea* resulted in significant changes in the defence proteome especially in early time points.

5.3 Responses of *Arabidopsis* to infection by *B. cinerea*

The interactions between pathogens and their potential hosts involve close communications in which pathogens constantly devise ways of exploiting nutrient resources of their hosts. For instance *B. cinerea* has evolved and employs various pathogenicity factors to promote host colonization (Comménil *et al.*, 1998; Collado *et al.*, 2000; Govrin and Levine, 2000; ten Have *et al.*, 2001; Schouten *et al.*, 2002; Valette-Collet *et al.*, 2003; Espino *et al.*, 2005). Plants on the other hand have evolved mechanisms to recognize presence of pathogens and respond by deploying various antimicrobial defences. Some of these defences also referred to as preformed or passive do exist constitutively whether the plant is infected or not and hence provide the first line of defence while others are activated following pathogen recognition and are referred to as inducible (Mysore and Ryu, 2004; Nurnberger and Lipka, 2005). Constitutive defences provide immediate protection but there is a trade-off penalty with regard to growth and fitness of the plant while inducible defences are cheaper since they are only induced upon pathogen recognition (Baldwin, 1998).

5.3.1 The role of the cell wall in host resistance

Compounds such as the cuticle and wax which are present on the surface of the leaf provide the first line of defence and have been shown to be important in host resistance. The plant cell wall offers the second line of defence and has been perfectly designed to achieve this function. It is composed of compounds (e.g. cellulose, hemicellulose, pectin, lignin and proteins) which form a structure hard to physically break and needs a variety of enzymes for dissolution (Vorwerk *et al.*, 2004). *B. cinerea* has evolved enzymes referred to as cell wall degrading enzymes (CWDEs). These enzymes were extensively described in Chapter 1. These enzymes are of various types and are designed to degrade various components of the cell wall. They are also designed for different plants and are active under different conditions such as pH (Wubben *et al.*, 2000; ten Have *et al.*, 2001). All these factors enable *B. cinerea* to colonize a broad range of hosts. Plants on the other hand have evolved proteins aimed at counteracting the activity of some of these enzymes such as the

polygalacturonase inhibiting proteins (PGIPs). These proteins are localized in the cell wall and target endopolygalacturonases produced by various fungi (Cervone *et al.*, 1990). The *Arabidopsis* genome contains two PGIP encoding genes (*AtPGIP1*, *At5g06860* and *AtPGIP2*, *At5g06870*). Both these genes where expressed following infection by *B. cinerea* however, *AtPGIP1* was induced very early in the interaction and cluster analysis also demonstrated that it is also it is also induced by other fungal pathogens except the biotrophic *E. orontii*. These proteins especially *AtPGIP1* therefore provide potential candidates for enhancing resistance in susceptible hosts. This supposition is based on studies demonstrating that transgenic plants expressing a pear PGIP showed reduced fungal colonization (Powell *et al.*, 2000; Agüero *et al.*, 2005).

5.3.2 Phytohormones in host-pathogen interactions

Although plants lack an efficient transport and central nervous system like animals, they still regulate a number of cellular process which include growth, ripening, senescence, response to adverse environmental conditions and many others. This is made possible by a multitude of phytohormones such as abscisic acid (ABA), auxins especially IAA, cytokinins, ethylene (ET), gibberellins, jasmonates (JA) and salicylates (SA) (Bartel, 1997; Leung and Giraudat, 1998; Reymond and Farmer, 1998; Chen *et al.*, 2005; Li and Guo, 2007). Some of these hormones such as ET, JA and SA are induced in response to pathogen infection and their role in mediating host defence responses to pathogens has been elaborated (Lund *et al.*, 1998; Reymond and Farmer, 1998; Dempsey *et al.*, 1999). In this study, there were indications of the presence above mentioned phytohormones especially ABA, ET, IAA, JA, and SA. The presence of ET, JA and SA was expected as these hormones have been shown to have direct role in host resistance to pathogens.

However, the presence of ABA and IAA raises a number of question as to what the role of these hormones could be. Plants produce ABA if subjected to abiotic stresses but it seems to have been produced following a biotic stress. The ABA produced in this interaction could have originated from a number of sources. It could have been produced in the host as a result of primary or secondary effects of infection or directly by the *B. cinerea* since it has also been shown to produce

this phytohormone even though its role in pathogenesis has not been elucidated. The speculation that ABA could have originated from *B. cinerea* is supported by a number of studies which have shown that application of this phytohormone enhances host susceptibility to pathogens (Edwards, 1983; Ward *et al.*, 1989; Audenaert *et al.*, 2002; Mohr and Cahill, 2003). It is also possible that *B. cinerea* manipulates ABA biosynthesis and signalling as a strategy to suppress host-defence responses as in the case of *P. syringae* pv. *tomato* (Torres-Zabala *et al.*, 2007).

Auxins especially IAA are known to be involved in different roles including host resistance in plants. Auxin signalling seems to have different roles in pathogenesis in different pathogens. Auxins seem to enhance host resistance to fungal pathogens including necrotrophs (Ueno *et al.*, 2004; Llorentea *et al.*, 2008) but on the contrary enhance host susceptibility to bacterial pathogens (Glickmann *et al.*, 1998; Navarro *et al.*, 2006). It is therefore possible that IAA may be produced in *Arabidopsis* to enhance its resistance to *B. cinerea* and this pathway may be a useful target for breeding programs.

5.3.3 Role of ET, JA and SA in host resistance

SA is known to mediate inducible defences effective against biotrophic pathogens in *Arabidopsis* (Dong, 1998; Glazebrook, 2001). The involvement of SA-mediated defence responses in resistance against *B. cinerea* was clearly observed as some of the *Arabidopsis* genes that were induced are also known to be induced by SA. The PAL pathway which was recently shown to be necessary for the synthesis of SA necessary for local resistance (Ferrari *et al.*, 2003) was also shown to be active as genes involved in this pathway were up-regulated close to the lesion. JA and ET are known to regulate inducible defences effective against necrotrophic pathogens (Dong, 1998; Glazebrook, 2001). The JA/ET-mediated responses play a vital role in both local and systemic resistance to *B. cinerea* hence the up-regulation of genes involved in the biosynthesis of these two hormones is a clear manifestation of their importance in this interaction (Vijayan *et al.*, 1998). The up-regulation of a number of genes known to require the two hormones for induction such as *PDF1.2* and *ERF1* further demonstrated the role of the two hormones in resistance of *Arabidopsis* to *B. cinerea* (Lorenzo *et al.*, 2003). The JA signalling pathway could also be the main reason why

expression profiles of *B. cinerea*, *P. infestans* and the insect pests especially the green peach aphid *M. persicae* were similar. This pathway has been shown to be essential in mediating host resistance to these organisms. The implication of these results is that it is possible to develop varieties that are resistant to multiple pathogens and pests by employing cluster analysis of various microarray experiments.

5.3.4 Secondary metabolism in host defence

Secondary metabolites may not be essential for plant growth but make it possible for the plant to survival in its environment (Kliebenstein, 2004). They are an important component of host defence responses to pathogen and herbivorous insect attack. Some of them are preformed such as the phytoanticipins which are present before infection by pathogens or are produced from pre-existing precursors following infection while others such as the alkaloids, glucosinolates (e.g. tryptophan, methionine and phenylalanine), phenylpropanoids (e.g. flavanols, anthocyanins, catechins an sinapates), phytoalexins (e.g. camalexin) and terpenoids (e.g. mono, di and sesquiterpenoids) are synthesized following pathogen infection or wounding by insect pests (Kliebenstein *et al.*, 2001; Kliebenstein, 2004). The role of some of these compounds is to restrict host invasion by pathogens while others such as the alkaloids can exterminate pathogens (Wittstock and Gershenzon, 2002). The terpenoids seem to be very important in host resistance to *B. cinerea*. These metabolites in addition to those closely related to them such as the sesquiterpenoids and sterols have been shown to be important factors in resistance to several insect pests and pathogens even though more examples of plant terpenes are involved in insect resistance than against pathogens (Bennett and Wallsgrove, 1994; Aharoni *et al.*, 2003). The similarity in host defence responses to necrotrophs like *B. cinerea* and insects could explain the induction of genes encoding the enzymes involved in the production of terpenoids.

5.4 Future studies

This work has enriched our understanding on the defence responses employed by *Arabidopsis* to resist infection by *B. cinerea* however, to gain an in-depth understanding of this pathosystem, more work needs to be done especially in areas of Spatial gene expression profiling, functional genomics, expression proteomics and quantitative trait loci (QTL) mapping.

5.4.1 Spatial gene expression profiling

Camalexin extraction and AOS were shown to accumulate before 12 hrs following inoculation while proteomic expression profiling (this study) demonstrated that host response occur before 6 hrs. Other studies have also demonstrated the production of metabolites such as camalexin, glucosinolates, sinapates and SA close and away from the developing lesion. This spatial expression was demonstrated but at a transcriptome level in a microarray experiment. However, comparison of the spatial and temporal microarray experiments demonstrated limited overlap an indication that some of the genes were absent in either experiment. It is therefore worth performing the spatial experiment for many replications over a time course commencing before 6 hrs to investigate how expression profiles of spatially expressed genes change over time during lesion development. It is possible that there are genes expressed very early within the vicinity of the developing lesion and encode compounds whose main role is to impede the increased development of the fungus. This information will be very important not only in enhancing our knowledge on this pathosystem but also its application in developing crop varieties that can tolerate this pathogen and other pathogens as observed in cluster analysis. Secondly, by applying laser microdissection, it will also be possible to look at a much higher spatial resolution.

5.4.2 Functional genomics

Microarray studies provide clues on the probable function of genes in many biological systems. However, the actual role of the identified genes needs to be thoroughly studied. In this study, we employed a reverse genetics technique of insertional mutagenesis using T-DNA insertional lines on a subset of genes however, the success

rate was very low, an effect that can be attributed to redundancy or gene induction through secondary effects. Two options may be employed to get around the effect of redundancy and one of them is generation of RNAi lines (Wesley *et al.*, 2001). In RNA interference, the gene under study is amplified and cloned into the vector with two identical copies in opposite orientations. The construct is then introduced into the plant. The Double stranded RNA molecule resulting from this construct and corresponding to the gene sequence will cause silencing of the endogenous copy of that gene. The advantage with using RNAi lines is that they can be designed to knock out the whole family of genes. A large stock of *Arabidopsis* RNAi knock-outs developed by AGRIKOLA (Hilson *et al.*, 2004) is available from Nottingham Arabidopsis Stock Center.

The second option is to overexpress candidate genes in transgenic lines and assess them for increased resistance. Lack of altered resistance suggests no role in resistance while an increase in resistance would suggest a role in resistance. Potential candidates for assessing for altered susceptibility (knock-out mutants) or resistance (overexpressors) include genes encoding transcription factors (e.g. WRKY and MYB), ABC family transporters, protein kinases and those involved in secondary metabolism, especially the terpenoids. Cluster analysis demonstrated that there was a significant overlap between *Arabidopsis* genes induced *B. cinerea* and those induced by other fungal pathogens. It is therefore necessary that confirmed transgenic lines are tested for altered resistance/susceptibility to known other *Arabidopsis* pathogens such as *Fusarium oxysporum*, *Erysiphe orontii*, *Pseudomonas syringae*, *Xanthomonas campestris*, *Hyaloperonospora parasitica*, *C. carbonum*, *P. infestans* and *Erwinia amylovora*.

5.4.3 Expression proteomics

Transcriptomic studies with microarrays avail enormous and valuable information on the global changes in gene expression that take place in an organism following its exposure to a particular stress. This provides an insight on the defensive mechanisms deployed by that organism in response to the stress in question. However, because proteins control and mediate most of the biological activities in cells, proteomic studies provide a deeper understanding of transcriptomic information which

is very important in downstream applications of transcriptomic studies such as plant breeding. A number of techniques are available for studying proteomes of organisms. In this study, the 2D SDS PAGE and ProteomeLab PF2D were used however, ProteomeLab PF2D system, did not identify as much changes in absorbance of fractions and consequently as many proteins in these fractions as had been earlier envisaged. This could have been due to a number of reasons however, the most probable ones possibly were the inability of the extraction protocol to release most of the proteins and the masking effect of down-regulated genes especially RuBisCO. It is necessary that protocols for extracting plants proteins for ProteomeLab PF2D are optimized to identify one that results in fractionation of a higher number of them. Secondly, depletion of RuBisCO will enhance both the resolving power and sensitivity of the system. The other technique that may be used to analyze the *Arabidopsis* defence proteome after *B. cinerea* infection is iTRAQ. Its advantages over ProteomeLab PF2D include the ability to analyze more (four) samples concurrently and involves less chromatography steps. In addition to studying the proteome that is expressed or repressed following infection, it will also be necessary to investigate the proteome that undergoes post-translational modifications especially phosphorylation and glycosylation as a number of protein kinases and UDP-glucosyl transferases were significantly up-regulated respectively. Lastly, this study identified only abundant and not low abundant proteins especially the protein kinases and transcription factors yet these proteins are very important in understanding signal transduction. Besides depletion of RuBisCO, these low abundant proteins may be enriched first through protein prefractionation using reversed-phase liquid chromatography.

5.4.4 Quantitative trait loci mapping

Disease resistance is one of the traits known to be influenced by many genes each segregating according to Mendelian laws (Lander and Botstein, 1989; Zeng, 1994). These traits are referred to as continuous or quantitative traits while the loci underlying them are referred to as quantitative trait loci (QTL) (Doerge, 2002; Jansen, 2003). The genetic variation that underlies quantitative traits results from the segregation of numerous QTLs each explaining a portion of the total genetic variation (Mackay, 2001; Glazier *et al.*, 2002; Paran and Zamir, 2003). Expression of QTLs is

modified by interaction that occur within them and by the environment (Paran and Zamir, 2003). The statistical study of alleles that occur in a loci and the phenotypes that they produce is referred to as QTL mapping. QTL mapping studies are aimed at determining either, the loci responsible for the variation in the quantitative trait, or the number, location and interaction of these loci, or the actual genes and their functions (Borevitz and Chory, 2004).

In plants, QTL mapping can be carried out using backcross (BC) populations (Shappley *et al.*, 1998), double haploids (DH) Yan *et al.* (1998), F_2 populations (Simko *et al.*, 1999), and recombinant inbred line (RILs) (Lister and Dean, 1993). RILs give a more precise assessment of the QTL properties especially for low heritability traits since they are inbred and not heterozygous (Wu *et al.*, 2003). They also undergo multiple rounds of meiosis before homozygosity can be reached, hence closely linked genes easily recombine. Denby and associates (2004) used 100 RILs developed by Lister and Dean (1993) and identified broad QTL governing resistance to *B. cinerea* however, the genes underlying these QTLs are not known. Because each QTL covers a fairly large portion, it is necessary to narrow down the region defining each QTL by increasing the number of RILs possibly the complete set of 300 RILs. Following this fine scale mapping, the location of up-regulated genes identified in the microarray study can then be compared to the location of the known QTL to investigate whether any of these genes explain the underlying resistance QTL.

The other option would be to develop a mapping population or use existing mapping populations such as the *Arabidopsis* RIL population used by Denby *et al.* (2004). Each individual within the mapping population is subjected to global gene expression profiling using microarrays to identify genes that change after infection with *B. cinerea.* Data from each individual can then be statistically analyzed to identify expression QTLs (eQTLs) that control the expression of each gene. The location of eQTLs from the individual analyses for all the genes are then superimposed to identify common regions that control the expression of a large number of genes (Schadt *et al.*, 2003; Kliebenstein *et al.*, 2006; Göring *et al.*, 2007). Because phenotypic differences between individuals is thought to be dependent on quantitative differences in gene expression, the identification of eQTLs will enhance our understanding of the mechanisms of resistance in *Arabidopsis* to *B. cinerea.* Chro-

mosomal regions containing major eQTLs controlling multiple genes also provide novel candidates for map-based cloning (Salvi and Tuberosa, 2005; Jordan *et al.*, 2007).

5.5 Conclusion

Successful management of plant diseases calls for a holistic approach in which all factors that may play a role are put into consideration. For this to be achieved we need to have a comprehensive understanding of these factors. For instance, what initiates epidemics? How do they explode? What factors promote spread? What are the most important factors in the pathogenesis process? Are natural sources of resistance available? Fortunately, for *B. cinerea*, most of the information concerning aetiology, epidemiology and pathogenesis of this pathogen is available and a lot of research is still in progress. This study focussed on identifying sources of resistance to *B. cinerea* in *Arabidopsis* using microarrays and candidate genes have been successfully identified. Integrating this information about host resistance and information concerning pathogenicity of *B. cinerea* will be very useful in developing practices that can offer the best protection against the grey mold fungus in vulnerable crops.

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Glossary

Alternative splicing The RNA splicing variation mechanism in which the exons of the primary gene transcript, the pre-mRNA, are separated and reconnected so as to produce alternative ribonucleotide arrangements.

Anamorph The asexual reproductive stage also referred to as the imperfect stage.

Aneuploidy A change in the number of chromosomes that can lead to a chromosomal disorder.

Apothecium A fruiting body where the asci are born in a single, orderly layer on an open, fairly flat surface, **pl. Apothecia**.

Appressorium An enlarged fungal filament that adheres to the surface of the host, prior to penetration.

Arithmetic mean The mean calculated by adding a set of values and then dividing the sum by the number of values.

Ascospore A spore contained in an ascus.

Ascus The sexual spore-bearing cell produced in ascomycete fungi, **pl. Asci**.

Avirulent Lacking the ability to cause disease.

Biological control The reduction of pest populations by use of natural enemies and compounds.

Biotrophic An organism that obtains nutrients from living host cells without killing them.

Calmodulin A small cytosolic regulatory protein that binds four Ca^{2+} ions. The Ca^{2+}/calmodulin complex binds to many proteins thereby activating or inhibiting them.

Chromatofocusing A column chromatographic procedure for separating proteins according to their isoelectric point.

Coefficient of variation The ratio of standard deviation to the mean. It is a measure of dispersion of a probability distribution.

Complementary DNA, cDNA A DNA molecule copied from an mRNA molecule by reverse transcriptase.

Conidiation The biological process in which filamentous fungi asexually form spores.

Conidiogenesis The mode of conidium formation.

Conidiophore A specialized hypha on which one or several conidia are produced.

Conidium An asexually produced fungal spore, formed on a conidiophore, **pl. Conidia**.

Constitutive Constantly present, whether there is demand or not.

Corroliferous monocotyledons Monocotyledonous plants that produce a bulb. Examples include those belonging to the Alliaceae such as onions, leek, and garlic and Liliaceae such as lily and tulip.

Cycle threshold The cycle threshold (Ct) value is the number of cycles needed for the fluorescence signal to reach a specific threshold level of detection and is inversely correlated with the amount of template nucleic acid present in the reaction.

Cyclic AMP, cAMP A second messenger produced in response to hormonal stimulation of certain GPCRs that activates protein kinase A.

Damping-off The collapse and rot of seedlings near soil level before emergence or soon after emergence.

Dikaryon A hyphal compartment, mycelium or fungal cell occupied by a pair or pairs of closely associated, genetically different, sexually compatible nuclei.

Disease severity The measure of damage done by a disease.

Effectors Microbe-derived molecules whose intrinsic activities are to interact with and affect the host.

Elicitor A molecule produced by a pathogen, inducing a response in the host. Conversely, an elicitor can be produced by the host which induces a response by the pathogen.

False Discovery Rate A statistical method used in multiple hypothesis testing to correct for multiple comparisons. It controls the expected proportion of incorrectly rejected null hypotheses (type I errors) in a list of rejected hypotheses.

G protein-coupled receptors, GPCRs A large protein family of transmembrane receptors that sense molecules outside the cell and activate inside signal transduction pathways and, ultimately, cellular responses. They are also referred to as seven transmembrane receptors, heptahelical receptors, and G protein linked receptors (GPLR).

Gene expression Conversion of the information encoded in a gene via transcription and translation, resulting in the production of a protein and the appearance of the phenotype determined by that gene.

Genealogy The study and tracing of family pedigrees.

Geometric mean The mean calculated as the n^{th} root of the product of n values.

Guanine nucleotide binding proteins, G proteins, A family of proteins involved in second messenger cascades.

Heamocytometer A device used to count cells and many other types of microscopic particles like fungal spores.

Heterokaryosis The presence in a cell of two or more nuclei of different genetic origin. It occurs naturally in some fungi when cells fuse but their nuclei do not.

Heterothallic Fungi producing compatible male and female gametes on physiologically distinct mycelia.

Host A plant that is invaded by a parasite and from which the parasite obtains its nutrient.

Host range The range of plants in which a pathogen is capable of causing disease.

Housekeeping gene A gene that encodes a product required in the maintenance of basic cellular processes or cell architecture.

Hypersensitive response An early inducible defence response elicited by pathogen-specific avirulence determinants and causes necrosis and cell death to restrict the growth of pathogens.

Hypersensitivity pneumonitis Inflammation of the peripheral airways and surrounding interstitial tissue caused by inhalation of allergens such as small airborne particles.

Hyphae A single tubular thread-like filament of a fungal mycelium. The hypha is the basic structural unit of a fungus, **pl. Hypha**.

In situ hybridization Any technique for detecting specific DNA or RNA sequences in cells and tissues by treating samples with single-stranded RNA or DNA probes that hybridize with the sequence of interest.

Inoculate To bring a pathogen into contact with a host plant or plant organ.

Isoelectric point, pI The pH of a solution at which a dissolved protein has a net charge of zero and therefore does not move in an electric field.

Isolate A single spore or culture and the subcultures derived from it.

Latent infection Where the host is infected with a pathogen but does not show any symptoms.

Log ratio The logarithm, usually to the base 2, of the ratio of the measured signal intensities in the two channels of a two-colour microarray experiment.

Melting curve analysis The melting point of double-stranded DNA is the temperature at which 50% of the DNA is single stranded and this temperature is dependent on DNA length and GC content. When using SYBR Green I, a sudden decrease in fluorescence is detected when the melting temperature is reached.

Messenger RNA, mRNA Any RNA that specifies the order of amino acids in a protein.

Microarrays A collection of microscopic DNA spots, commonly representing single genes, arrayed on a solid surface by covalent attachment to a chemical matrix.

Microbe-associated Molecular Pattern, MIMPs A structural element from within a molecule of a potential pathogen.

Microbe-induced Molecular Pattern, MIMP The product of an intrinsic activity of an effector.

Mitogen activated protein kinase, MAP kinase A protein kinase that is activated in response to cell stimulation by many different growth factors and that mediates cellular responses by phosphorylating specific transcription factors and other target proteins.

Motif A short conserved structure that often can be recognized in the primary amino acid sequence in a protein.

Mutation Changes to the base pair sequence of the genetic material of an organism.

Mycelium A mass of hyphae that forms the body (thallus) of a fungus, **pl. Mycelia**.

Necrotroph An organism that causes the death of host tissues as it grows through them such that it is always colonizing dead substrate.

Non-pathogenic Incapable of causing disease.

Oligonucleotide A short, single-stranded DNA that is usually synthesized *in vitro* and often used as a probe in hybridizations or as primers for the polymerase chain reaction.

Oxidative burst The rapid production of active oxygen species (superoxide radical and hydrogen peroxide) triggered by elicitor molecules following invasion by pathogenic organisms.

Oxidative stress An imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage.

Parametric test A statistical test in which assumptions are made about the underlying distribution of observed data.

Parasexuality A phenomenon where two cell nuclei merge without any sexual process and the chromosome count is doubled.

Pathogen A disease causing organism or agent.

Pathogen-associated molecular patterns, PAMPs Small molecular motifs consistently found on pathogens recognized by toll-like receptors and other pattern recognition receptors in plants and animals.

Pathogenesis The sequence of processes in disease development that describes a pathogen's association with its host.

Pathogenicity The ability to cause disease.

Perithecium A flask-shaped fruiting-body produced by some species of the Ascomycota; from the neck of which asci are discharged, **pl. Perithecia**.

Photoperiod The duration of an organism's daily exposure to light, considered especially with regard to the effect of such exposure on growth and development

Phytoalexin A substance produced in higher plants in response to a number of stimuli (chemical, physical or biological) that inhibits the development of a microorganism.

Phytoanticipin A substances synthesized by the plant at a constant rate and therefore always present in the tissues of the plant.

Polymerase chain reaction, PCR A technique for amplifying a specific DNA segment in a complex mixture by multiple cycles of DNA synthesis from short oligonucleotide primers followed by brief heat treatment to separate complementary strands.

Primer A short nucleic acid sequence containing a free 3'-hydroxyl group that forms base pairs with a complementary template strand and functions as the starting point for addition of nucleotides to copy the template strand.

Probe The DNA hybridized to the array or the mobile substrate.

Protein kinase A A cytosolic protein kinase that is activated by cAMP and functions to phosphorylate and thus regulate the activity of numerous proteins.

RNA interference Functional inactivation of a specific gene by experimental introduction of a corresponding double stranded RNA, which induces degradation of the complementary single-stranded mRNA encoded by the gene but not that of mRNAs with a different sequence.

Sclerotium A hard, resistant vegetative resting body of a fungus composed of a compact mass of hyphae and capable of surviving under unfavourable environmental conditions. Under favourable conditions the sclerotium can produce sexual or asexual fruiting bodies, **pl. Sclerotia**.

Secondary metabolite A compound that is not necessary for growth or maintenance of cellular functions but is, in general, synthesized for the protection of a cell or microorganism during the stationary phase of its growth cycle.

Spore A specialized reproductive body in fungi (and some other organisms), containing one or more cells, capable of developing into an adult.

Standard curve A curve consisting of cycle threshold or crossing point values plotted against the log of standard concentrations from which the concentration or quantity of unknown samples is determined.

Standard deviation The square root of the variance. It measures spread of values in a random variable or population.

Target The DNA spotted on the array or the immobile substrate.

Teleomorph The sexual reproductive stage also referred to as the perfect stage.

Toxin A poisonous substance of biological origin.

Transcription The process whereby a base sequence of mRNA is synthesized on a complementary segment of DNA.

Transcriptome The set of all mRNA molecules produced in one or a population of cells.

Translation The process that occurs at the ribosome whereby the information in mRNA is used to assemble amino acids into a protein.

Transposable elements Sequences of DNA that can move around to different positions within the genome of a single cell. They are also referred to as **transposons** and the process **transposition**.

Virulence The degree of pathogenicity of a microbe or its relative ability to cause disease.

Appendices

Appendix A

Microarray Time course Experiment

A.1 *Arabidopsis* genes significantly up-regulated at 12 hpi after treatment of *Arabidopsis* leaf tissue with *Botrytis cinerea*. The experiment was replicated five times (Rep 1-5) and only genes significantly induced more than 2 fold (log of 1) on average are shown.

Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At4g20200	5.06	5.91	5.08			5.35	terpene synthase
At1g10585	4.91	3.02		3.42	6.33	4.42	expressed protein
At2g43620	4.80			2.60	5.66	4.35	chitinase
At4g12500	5.05	4.88	1.95		5.38	4.32	protease inhibitor/seed storage/LTP
At5g61160	3.47	4.62	2.16		6.17	4.10	transferase family protein
At3g04720	3.29	3.09	0.41	6.58	7.09	4.09	hevein-like protein (HEL)
At2g28210	2.70	3.16		5.31	4.98	4.04	carbonic anhydrase
At1g54100	3.90	3.64		4.29	4.27	4.03	aldehyde dehydrogenase
At5g49780	4.51	6.45	1.08			4.01	leucine-rich repeat transmembrane protein kinase
At1g26420	3.45	3.82		2.23	6.34	3.96	FAD-binding domain-containing protein
At2g30750	3.87	4.24	0.72	4.41	6.41	3.93	cytochrome P450 71A12
At2g39030	3.47	2.20		4.52	5.35	3.88	GCN5-related N-acetyltransferase
At4g08780	2.60	3.50		3.71	5.26	3.77	peroxidase
At1g26400	1.91	4.53	0.80	5.27	6.16	3.74	FAD-binding domain-containing protein
At3g58850	3.78	4.24	3.18			3.73	expressed protein
At3g45300		2.91		2.67	5.22	3.60	isovaleryl-CoA-dehydrogenase
At2g29350	3.61	2.43		4.54	3.62	3.55	tropinone reductase
At1g05700	4.62	4.78	1.18	2.58	4.50	3.53	leucine-rich repeat protein kinase
At1g02930	3.96	4.56	1.81	2.75	4.28	3.47	glutathione S-transferase
At2g30770	2.96	4.71	0.35	4.15	5.14	3.46	cytochrome P450 71A13
At4g15610	3.24	2.27		3.48	4.76	3.44	integral membrane family protein
At4g12490	4.45	4.67	1.47	2.51	4.09	3.44	protease inhibitor/seed storage/LTP
At5g06730	5.81	4.21	2.78	0.88		3.42	peroxidase
At3g54640	3.47	3.88	1.40	3.28	4.95	3.40	tryptophan synthase, alpha subunit
At1g02920	4.06	4.27	0.83	3.09	4.68	3.39	glutathione S-transferase
At4g35770	4.32	3.94	-0.41	3.98	5.11	3.39	senescence-associated protein (SEN1)
At4g33150	3.43	2.99	2.68	4.06		3.29	lysine-ketoglutarate reductase
At1g15520	2.81	3.49	0.53	4.39	5.16	3.28	ABC transporter family protein
At2g36800	4.42	5.75	3.69	2.12	0.43	3.28	UDP-glucosyl transferase
At2g43510	4.38	3.41	-0.66	4.48	4.16	3.16	trypsin inhibitor
At3g21520	3.10	3.89		2.80	2.70	3.12	expressed protein
At1g21475	3.61	1.81	0.97	5.30	3.79	3.10	hypothetical protein
At1g10070	1.67	2.29	0.61	4.60	6.28	3.09	branched-chain amino acid aminotransferase 2
At5g38900	2.44	3.82	0.29	3.59	5.24	3.07	DSBA oxidoreductase
At1g26380	3.22	3.03	0.59	3.70	4.76	3.06	FAD-binding domain-containing protein
At1g61800	2.83	4.17	0.37	2.96	4.89	3.05	glucose-6-phosphate/phosphate translocator
At2g45220	1.24	2.08	2.02	4.66	4.87	2.97	pectinesterase family protein
At4g32140	3.02	3.08	2.75			2.95	expressed protein
At2g26560	3.05	3.36	0.01	3.15	5.02	2.92	patatin
At3g59930	3.15	1.93	1.62	5.72	1.77	2.84	expressed protein
At1g52200	3.28	1.76		2.14	4.15	2.83	expressed protein
At1g32350	2.18	1.77	0.66	4.10	5.24	2.79	alternative oxidase
At4g14130	3.47	4.08	0.73			2.76	xyloglucan:xyloglucosyl transferase
At3g25250	3.76	4.38	2.69	0.73	2.10	2.73	protein kinase family protein
At5g07010	2.09	2.42	0.20	3.67	5.25	2.73	sulfotransferase family protein
At3g55240	4.37	3.45	0.36			2.72	expressed protein
At4g37980	3.99	2.09	1.03	2.83	3.56	2.70	mannitol dehydrogenase
At2g35980	1.66	2.41	0.48	4.05	4.66	2.65	harpin-induced family protein
At3g49620	1.56	1.94	0.37	4.26	5.04	2.63	2-oxoacid-dependent oxidase
At3g49110	2.03	3.14	0.17	3.31	4.46	2.62	peroxidase 33
At1g11610	0.25	2.71	1.12	4.42	4.50	2.60	cytochrome P450
At5g39610	3.65	3.45	0.66			2.59	no apical meristem (NAM) family protein
At2g24180	2.45	1.02		2.92	3.81	2.55	cytochrome P450 family protein
At1g54575	3.56	1.53	0.66	3.25	3.69	2.54	expressed protein
At5g14780	3.08	2.53	0.72	3.10	3.27	2.54	formate dehydrogenase
At3g53600		1.99	0.73	2.44	5.01	2.54	Zinc finger (C2H2 type) protein
At1g59535	2.01	1.39		3.33	3.39	2.53	hypothetical protein
At3g49120	2.64	2.8	1.35	2.66	3.05	2.50	peroxidase
At5g07000	2.72	3.27	0.44	3.47	2.62	2.50	sulfotransferase family protein
At1g62380	1.19	1.41	1.06	4.54	4.12	2.47	1-aminocyclopropane-1-carboxylate oxidase
At1g51890	2.94	1.78		1.79	3.35	2.46	Leucine-rich repeat protein kinase
At5g57890	2.66	3.74	0.23	1.93	3.61	2.43	anthranilate synthase beta subunit
At1g24909	3.20	2.61	0.53	2.09	3.68	2.42	anthranilate synthase beta subunit
At1g52890	3.07	2.42	1.19	1.79	3.63	2.42	no apical meristem (NAM) family protein
At4g12470	2.75	2.59	0.57	2.12	3.85	2.37	protease inhibitor/seed storage/LTP
At1g25220	2.95	2.80	0.60	1.61	3.73	2.34	anthranilate synthase beta subunit
At1g21310	1.90	2.32	0.34	3.83	3.31	2.34	proline-rich extensin-like family protein
At3g50770	0.85	1.34	1.63	2.67	5.16	2.33	calmodulin-related protein
At3g03470	3.25	2.09	0.92	3.34	2.01	2.32	cytochrome P450
At1g03905	2.40	3.25	1.06	2.54		2.31	ABC transporter family protein
At5g14940	2.09	1.29		3.54		2.31	proton-dependent oligopeptide transport
At2g02930	2.69	2.42	0.81	2.42	3.18	2.30	glutathione S-transferase
At1g62300	1.34	1.49	0.84	3.55	4.18	2.28	WRKY family transcription factor

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At3g50210	1.33	2.38	0.54	2.72	4.36	2.27	2-oxoacid-dependent oxidase
At5g34870	4.00	1.89	0.83			2.24	zinc knuckle (CCHC-type) protein
At4g16146	3.00	1.20	0.81	2.21	3.91	2.23	expressed protein
At4g02520	2.47	1.85	0.03	2.45	4.24	2.21	glutathione S-transferase
At1g05010	3.61	2.21	0.81	1.10	3.20	2.18	1-aminocyclopropane-1-carboxylate oxidase
At5g11670	3.17	2.56	1.02	0.92	3.22	2.18	malate oxidoreductase
At5g67080	2.07	2.76	0.12	1.82	4.11	2.18	protein kinase family protein
At2g38465	3.18	1.72		1.12	2.65	2.17	expressed protein
At4g27450	1.28	3.35		1.86		2.16	expressed protein
At1g25083	2.00	2.46	0.55	2.03	3.59	2.12	anthranilate synthase beta subunit
At5g27760	1.85	2.31		1.24	3.02	2.10	hypoxia-responsive
At5g59220	2.38	1.24	0.81	2.50	3.40	2.07	protein phosphatase 2C
At3g57980	2.87	2.69	0.57			2.04	DNA-binding bromodomain-containing protein
At1g48070	1.84	2.10	1.06	1.94	3.27	2.04	expressed protein
At3g18080	2.67	1.50	0.63		3.32	2.03	glycosyl hydrolase family 1 protein
At5g26340	1.65	2.38	0.63	2.21	3.26	2.03	hexose transporter
At4g15530	2.12	1.77	0.25	2.65	3.36	2.03	pyruvate phosphate dikinase
At3g47410	0.83	1.56		2.34	3.25	1.99	hypothetical protein
At5g03730	2.66	1.98	0.51	1.61	3.00	1.95	serine/threonine protein kinase
At5g18130	2.01	1.52	0.27	3.81	2.11	1.94	expressed protein
At1g24807	1.32	2.18	1.18	1.85	3.08	1.92	anthranilate synthase beta subunit
At3g55870	2.06	2.54		1.54	1.54	1.92	anthranilate synthase, alpha subunit
At3g22200	2.01	1.01	0.51	2.60	3.43	1.91	4-aminobutyrate aminotransferase
At5g36270	3.30	1.97	0.36			1.88	dehydroascorbate reductase
At5g13600	2.21	3.25	2.93	0.80	0.18	1.87	phototropic-responsive NPH3
At5g54500	0.82	1.13	1.40	2.78	3.11	1.85	quinone reductase
At3g28930	2.03	1.84	0.63	1.32	3.38	1.84	avrRpt2-induced AIG2 protein
At4g30270	3.08	2.72	-0.16	2.12	1.47	1.84	MERI-5 protein
At1g20620	2.68	2.12	0.54	1.24	2.40	1.80	catalase 3 (SEN2)
At2g37130	1.65	1.71	0.57	2.99	2.11	1.80	peroxidase 21
At5g33290	1.28	1.33	0.38	2.33	3.61	1.79	exostosin family protein
At5g27380	1.43	1.57	0.80	1.44	3.60	1.77	Glutathione synthetase
At1g65240	1.65	2.47	0.84	1.69	2.17	1.76	aspartyl protease
At2g43570	1.74	1.28	0.68	2.20	2.91	1.76	chitinase
At5g39130	2.32	1.6	0.60	2.49		1.75	Germin-like protein
At1g18800	2.53	2.13	1.33		0.99	1.75	nucleosome assembly protein
At3g63270	3.10	1.45	0.49	1.90		1.74	expressed protein
At1g76930	1.47	1.27	0.09	2.93	2.87	1.73	proline-rich extensin-like family protein
At3g26740	2.99	1.96	0.06	1.42	2.17	1.72	light responsive protein-related
At5g67400	1.53	1.38	0.25	2.20	3.16	1.70	peroxidase 73
At2g28860	1.01	1.70	0.40	1.93	3.43	1.69	cytochrome P450 family protein
At2g16260	2.36	2.14	0.56			1.69	glycine-rich RNA-binding protein
At4g01897	1.94	3.49	0.85	1.10	1.03	1.68	expressed protein
At1g24996	1.84	2.31	0.80			1.65	expressed protein
At5g27980	1.55	1.42	1.99			1.65	seed maturation family protein
At3g21590	1.06	1.89			2.01	1.65	senescence/dehydration-associated
At3g15150	2.18	1.88	0.85			1.64	expressed protein
At3g11150	1.46	1.60	0.59	1.87	2.65	1.63	expressed protein
At5g01850	2.51	2.37	1.03	0.70	1.55	1.63	protein kinase
At1g26390	1.94	2.69	0.23			1.62	FAD-binding domain-containing protein
At5g14920	1.71	1.52	0.49	2.14	2.19	1.61	gibberellin-regulated
At5g54820	1.82	1.57	1.39			1.59	F-box family protein
At2g46680	2.11	1.34	0.86	2.31	1.26	1.58	homeobox-leucine zipper protein 7
At1g21245	2.30	1.71	0.72			1.58	Wall-associated kinase-related
At4g27070	0.95	1.63	0.43	1.79	3.06	1.57	tryptophan synthase, beta subunit 2
At1g29610	1.94	1.59	0.61	1.82	1.86	1.56	hypothetical protein
At2g42790	1.86	1.13	0.14	2.71	1.76	1.52	citrate synthase
At1g53070	1.40	2.29	0.09	1.31	2.51	1.52	legume lectin family protein
At2g18170	1.31	0.58	0.71	2.32	2.70	1.52	mitogen-activated protein kinase
At1g24430	1.34	1.71	0.61	1.52	2.42	1.52	transferase family protein
At2g18380	1.68	1.43	1.08		1.89	1.52	zinc finger (GATA type) protein
At3g46210	1.75	0.46	1.02	1.43	2.92	1.51	3' exoribonuclease
At4g05020	2.44	2.04	0.72	0.53	1.80	1.51	NADH dehydrogenase-related
At3g53160	0.97	0.75	1.04	1.99	2.82	1.51	UDP-glucosyl transferase
At3g51190	2.31	1.66	-0.13	1.67	2.00	1.50	60S ribosomal protein L8
At4g14020	2.20	1.29	0.33	1.82	1.77	1.48	rapid alkalinization factor
At5g57300	1.43	1.37	1.03	1.14	2.43	1.48	UbiE/COQ5 methyltransferase protein
At1g28230	1.41	1.82	1.15			1.46	purine permease
At5g43350	1.59	1.09	0.91	2.17		1.44	inorganic phosphate transporter
At1g49160	0.64	1.13	0.90	2.01	2.53	1.44	protein kinase family protein
At1g13360	2.08	0.91	0.66	0.75	2.77	1.43	expressed protein
At1g22400	1.01	2.21	0.05	1.33	2.55	1.43	UDP-glucosyl transferase
At1g54130	1.85	1.54	0.52	1.32	1.89	1.42	RelA/SpoT protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At1g53540	1.97	0.85	0.55		2.27	1.41	17.6 kDa class I small heat shock protein
At3g28940	2.66	1.13	0.60	0.56	2.10	1.41	avirulence-responsive protein
At3g04070	1.76	1.19	0.65	1.65	1.73	1.40	no apical meristem (NAM) family protein
At5g64370	1.34	0.36	0.74	2.86	1.66	1.39	beta-ureidopropionase
At3g51860	0.85	1.39	0.48	2.55	1.69	1.39	cation exchanger
At4g13090	2.18	1.93	-0.21	1.61	1.42	1.39	Xyloglucan:xyloglucosyl transferase
At1g47780	1.12	0.86	0.53	2.11	2.27	1.38	acyl-protein thioesterase-related
At3g29034	0.36	1.63	1.07	1.37	2.47	1.38	expressed protein
At3g02875	1.31	0.61		2.18		1.37	IAA-amino acid hydrolase 1
At4g37770	2.09	2.29	1.30	0.04	1.08	1.36	1-aminocyclopropane-1-carboxylate synthase
At2g33150	1.57	1.03	0.16	2.41	1.64	1.36	acetyl-CoA C-acyltransferase
At1g07190	1.28	2.42	0.38			1.36	hypothetical protein
At3g26160	2.02	0.65	0.36	2.08	1.66	1.35	cytochrome P450 family protein
At3g18390	0.63	1.51	1.92			1.35	expressed protein
At1g59700	1.72	1.78	0.53	1.91	0.80	1.35	glutathione S-transferase
At2g14170	1.33	1.09	0.79	2.18		1.35	methylmalonate-semialdehyde dehydrogenase
At1g30780	1.69	2.28	1.84	0.28	0.54	1.33	F-box family protein
At4g21380	1.40	0.65	0.32	1.98	2.29	1.33	S-locus protein kinase
At4g08470	2.28	1.60	0.20	1.00	1.45	1.31	mitogen-activated protein kinase
At2g47210	0.40	1.36	2.55	0.93		1.31	myb family transcription factor
At2g25000	0.91	1.40	1.62			1.31	WRKY family transcription factor
At5g45070	0.72	1.82	1.37			1.30	disease resistance protein
At3g21850	1.14	0.52	1.12	2.34		1.28	E3 ubiquitin ligase SCF complex
At3g51000	1.28	1.37	1.06	1.87	0.77	1.27	epoxide hydrolase
At1g09245	0.98	1.04	0.95	1.13	2.27	1.27	expressed protein
At1g36763	0.84	0.73	0.45	2.30	1.99	1.26	hypothetical protein
At4g21980	2.32	0.76	0.58	1.73	0.85	1.25	autophagy 8a
At2g31345	0.85	1.69	0.25	2.21		1.25	expressed protein
At5g47730		1.43	1.16	0.42	2.01	1.25	SEC14 cytosolic factor family protein
At1g73790	1.39	1.54	0.46	1.39	1.42	1.24	expressed protein
At5g32410	0.84	1.16	0.32	2.64		1.24	hypothetical protein
At5g06860	1.88	0.98	-0.03	1.57	1.75	1.23	polygalacturonase inhibiting protein 1
At1g31930	1.00	0.92	0.37	1.70	2.09	1.22	extra-large guanine nucleotide binding protein
At5g35560	1.27	0.75	1.10	1.49	1.46	1.21	DENN (AEX-3) domain-containing protein
At5g04250	0.92	1.03	1.68	0.62	1.78	1.21	OTU-like cysteine protease
At1g74640	0.42	0.32	1.50	1.56	2.22	1.20	expressed protein
At1g62500	1.84	1.03	0.50	1.39	1.23	1.20	protease inhibitor/seed storage/LTP
At1g73950	1.26	1.57	2.12	0.66	0.38	1.20	Zinc finger (C3HC4-type RING finger) protein
At5g05500	0.39	1.05	0.92	1.11	2.47	1.19	pollen Ole e 1 allergen and extensin
At3g28550	0.70	0.81	0.38	1.85	2.20	1.19	proline-rich extensin-like family protein
At4g16000	0.34	0.79	1.80	1.17	1.81	1.18	expressed protein
At1g23020	0.32	1.17	0.81	2.44		1.18	ferric-chelate reductase
At4g25260	1.17	1.02	1.35			1.18	invertase/pectin methylesterase inhibitor
At4g29010	0.78	1.55	0.13	1.92	1.49	1.17	abnormal inflorescence meristem 1
At4g06746	1.27	0.98	0.92	0.57	2.12	1.17	AP2 domain-containing transcription factor
At5g22270	0.83	1.44	0.19	1.11	2.28	1.17	expressed protein
At4g23370	1.01	1.56	0.12	1.57	1.59	1.17	hypothetical protein predicted proteins
At3g48260	1.62	1.50	0.39			1.17	protein kinase family protein
At4g14980	0.32	0.86	1.12	2.32		1.16	DC1 domain-containing protein
At5g03990	1.09	0.90	0.97	1.91	0.95	1.16	expressed protein predicted protein
At3g01880	2.11	0.64	0.73			1.16	hypothetical protein
At1g13890	1.10	1.38	0.35	1.86	1.12	1.16	SNAP25 homologous protein
At2g31990	1.30	2.22	-0.08			1.15	exostosin family protein
At4g19860	1.05	0.88	0.47	1.91	1.41	1.15	lecithin:cholesterol acyltransferase
At5g44575	1.56	0.91	0.16	1.8	1.25	1.14	expressed protein
At1g21520	1.25	1.55	0.07	1.63	1.21	1.14	expressed protein
At4g31380	1.11	0.96			1.36	1.14	hypothetical protein
At5g39440	1.36	1.25	0.80			1.14	Snf1-related protein kinase
At5g67480	1.31	1.77	0.56	0.2	1.87	1.14	TAZ zinc finger family protein
At2g41410	2.02	1.21	0.37	0.56	1.50	1.13	calmodulin
At1g45145	2.03	1.38	0.31	1.14	0.80	1.13	thioredoxin H-type 5
At5g37600	1.74	0.56	0.34	1.08	1.87	1.12	glutamine synthetase
At2g39310	1.95	1.25	1.33	0.4	0.65	1.12	jacalin lectin family protein
At4g25900	1.36	0.97	0.44	1.99	0.80	1.11	aldose 1-epimerase family protein
At3g55880	0.70	1.17	0.79	0.80	2.07	1.11	expressed protein
At4g04760	0.90	0.76	0.79	1.45	1.68	1.11	sugar transporter family protein
At2g07280	1.08	1.82	0.38			1.10	hypothetical protein
At5g57655	1.08	1.18	0.16	2.00	1.09	1.10	Xylose isomerase family protein
At1g15210	0.96	1.87	0.55		0.95	1.08	ABC transporter family protein
At4g30960	1.56	0.25	0.49	1.94	1.16	1.08	CBL-interacting protein kinase 6
At3g02270	0.89	0.70	0.39	2.06	1.33	1.08	eIF4-gamma/eIF5/eIF2-epsilon
At5g59000	2.09	0.34	0.80			1.08	Zinc finger (C3HC4-type RING finger) protein
At3g16150	1.11	0.75		1.43	1.00	1.07	L-asparaginase

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At2g26440	1.59	1.13	0.34		1.23	1.07	pectinesterase family protein
At5g14640	0.76	1.72	0.14	1.54	1.18	1.07	protein kinase family protein
At2g02990	0.16	1.15	1.33	1.22	1.50	1.07	ribonuclease 1
At1g66660	0.59	1.16	0.53	1.06	2.04	1.07	seven in absentia (SINA) protein
At2g05710	1.17	0.62	0.37	1.71	1.43	1.06	aconitate hydratase
At1g69830	0.66	1.63	1.21		0.73	1.06	alpha-amylase
At4g03580	1.12	0.72	0.84	1.61	1.03	1.06	hypothetical protein
At4g29190	1.87	1.20	0.03	0.77	1.44	1.06	zinc finger (CCCH-type) protein
At3g13610	0.11	0.82	1.29		1.98	1.05	oxidoreductase, 2OG-Fe(II) oxygenase
At1g71030	1.44	0.78	0.24	1.18	1.59	1.04	myb family transcription factor
At5g43280	0.71	0.65	0.68	1.59	1.52	1.03	enoyl-CoA hydratase/isomerase
At5g42635	1.63	0.82	0.33	0.69	1.69	1.03	glycine-rich protein
At3g45620	1.12	0.88	0.14	1.96		1.02	transducin family protein
At5g56190	0.51	0.81		1.15	1.59	1.02	WD-40 repeat family protein
At3g06690	0.95	0.50		1.70	0.90	1.01	acyl-CoA oxidase family
At3g13790	0.80	0.54	0.69	1.21	1.82	1.01	beta-fructosidase (BFRUCT1)
At5g51920	0.86	0.88	1.28			1.01	expressed protein
At4g24000	1.13	0.28	0.84	1.75		1.00	cellulose synthase
At1g29640	0.94	0.49	0.82	1.78	0.99	1.00	expressed protein
At1g23070	0.70	1.67	0.41	1.22		1.00	hypothetical protein
At1g31640	0.57	0.79	0.83	1.34	1.49	1.00	MADS-box protein-related

A.2 *Arabidopsis* genes significantly down-regulated at 12 hpi after treatment of *Arabidopsis* leaf tissue with *Botrytis cinerea*. The experiment was replicated five times (Rep 1-5) and only genes significantly repressed more than 2 fold (log of 1) on average are shown.

Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At1g65490	-2.86	-5.76	-3.54	-6.26	-9.41	-5.57	expressed protein
At5g38420	-3.85	-3.68	-3.00	-6.27	-6.01	-4.56	RuBisCO small subunit 2B
At5g38430	-3.73	-4.02	-3.13	-6.38	-4.62	-4.37	RuBisCO small subunit 1A
At4g37930	-3.91	-4.31	-2.19	-5.43	-5.39	-4.24	glycine hydroxymethyltransferase
At5g36700	-3.35	-4.06	-1.18	-4.87	-7.05	-4.10	phosphoglycolate phosphatase
At1g67090	-3.42	-3.11	-3.00	-5.87	-5.09	-4.10	RuBisCO small subunit 1A
At3g26060	-2.49	-4.99	-1.15	-5.00	-5.93	-3.91	peroxiredoxin Q
At5g45680	-2.72	-3.41	-1.83	-3.58	-7.86	-3.88	FK506-binding protein 1
At4g22880		-0.50	-3.67		-7.39	-3.85	leucoanthocyanidin dioxygenase
At5g23060	-2.83	-4.40	-1.03	-4.06	-6.84	-3.83	expressed protein
At1g12900	-3.43	-3.16	-1.71	-5.13	-5.57	-3.80	GAPA-2
At2g39730	-2.85	-4.40	-2.61	-3.64	-5.48	-3.80	RuBisCO activase
At1g72610	-2.67	-3.21	-2.74	-4.85	-5.48	-3.79	germin-like protein
At4g22570	-2.74	-5.59	-1.47	-4.22	-4.67	-3.74	adenine phosphoribosyltransferase
At5g35480	-0.69	-3.10		-4.71	-6.35	-3.71	expressed protein
At1g34170		-1.60	-1.76		-7.69	-3.68	transcriptional factor B3 family protein
At3g54050	-2.15	-3.03	-1.53	-5.43	-6.03	-3.63	fructose-1,6-bisphosphatase
At5g38410	-3.02	-3.63	-3.04	-4.52	-3.9	-3.62	RuBisCO small subunit 3B
At5g35630	-2.77	-3.92	-2.44	-4.09	-4.54	-3.55	glutamine synthetase
At3g62030	-3.10	-3.97	-1.47	-4.62	-4.49	-3.53	peptidyl-prolyl cis-trans isomerase
At1g32060	-2.94	-3.51	-2.18	-3.89	-5.08	-3.52	phosphoribulokinase (PRK)
At5g58260	-2.05	-3.06	-0.94	-8.01		-3.51	expressed protein
At1g75690	-2.42	-2.65	-1.68	-4.71	-6	-3.49	chaperone protein dnaJ-related
At4g25050	-3.34	-2.66	-2.46	-3.20	-5.54	-3.44	acyl carrier family protein
At1g42970	-2.12	-3.47	-2.24	-4.56	-4.81	-3.44	GAPB
At5g36790	-2.88	-3.61	-0.78	-4.48	-5.4	-3.43	phosphoglycolate phosphatase
At2g21330	-2.47	-3.19	-1.78	-4.09	-5.56	-3.42	fructose-bisphosphate aldolase
At5g41050	-1.68	-2.94	-5.55			-3.39	expressed protein
At1g32470	-3.06	-3.31	-0.85	-4.76	-4.93	-3.38	glycine cleavage system H protein
At3g56290	-3.87	-5.17	-1.94	-2.40		-3.35	expressed protein
At4g26530	-2.95	-2.81	-1.42	-4.24	-5.29	-3.34	fructose-bisphosphate aldolase
At3g52720	-4.14	-4.63	-1.05	-1.87	-4.97	-3.33	carbonic anhydrase
At2g01590	-2.40	-3.34	0.56	-8.13		-3.33	expressed protein
At3g51820	-2.53	-3.10	-1.26	-3.36	-6.1	-3.27	chlorophyll synthetase
At1g14150	-2.09	-3.98	-0.99	-3.47	-5.78	-3.26	Oxygen evolving enhancer 3
At1g78840	-3.29	-2.84	-1.68	-3.41	-5.06	-3.26	F-box family protein
At3g14415			-2.19	-2.78	-4.76	-3.25	(S)-2-hydroxy-acid oxidase
At1g20340	-2.13	-2.98	-2.12	-4.61	-4.34	-3.24	plastocyanin
At4g18370	-1.61	-2.14		-4.47	-4.72	-3.23	protease HhoA
At3g55330	-2.48	-2.94		-3.41	-4.01	-3.21	photosystem II reaction center
At5g02120	-2.26	-3.49	-0.98	-3.39	-5.69	-3.16	thylakoid membrane one helix protein
At2g05310	-2.38	-2.61	-1.86	-3.30	-5.49	-3.13	expressed protein
At2g04039	-2.35	-3.19	-1.04	-3.17	-5.81	-3.11	expressed protein
At2g42220	-2.30	-2.88	-2.48	-3.84	-4.01	-3.10	rhodanese-like
At4g28660	-1.51	-3.46	0.26	-4.12	-6.49	-3.06	photosystem II reaction centre W
At3g06070	-2.23	-2.93	-1.03	-3.13	-5.87	-3.04	expressed protein
At1g70760	-2.83	-3.06	-1.21	-4.02	-3.96	-3.02	inorganic carbon transport protein-related
At1g44575	-2.12	-2.49	-0.75	-4.36	-5.17	-2.98	photosystem II 22kDa protein
At5g43750	-1.68	-1.05	-0.96	-5.01	-6.16	-2.97	expressed protein
At4g16410	-1.82	-1.30	-1.51	-3.52	-6.7	-2.97	expressed protein
At1g19150	-2.13	-3.20	-0.75	-4.41	-4.37	-2.97	chlorophyll A-B binding protein
At1g20020	-1.78	-3.25	-1.56	-4.84	-3.4	-2.97	ferredoxin NADP(+) reductase
At1g01060	-5.24	-3.35	-2.36	-0.34	-3.54	-2.96	myb family transcription factor
At3g24430	-1.40	-1.63	-6.01	-2.74		-2.94	expressed protein
At2g34860	-1.96	-2.53	-3.06	-3.27	-3.78	-2.92	chaperone protein dnaJ-related
At3g23610	-2.59	-3.30	-2.23	-2.97	-3.51	-2.92	dual specincity protein phosphatase
At4g33010	-2.28	-2.60	-1.23	-4.00	-4.48	-2.92	glycine dehydrogenase
At1g35680	-2.02	-2.22	-1.29	-4.05	-4.88	-2.89	50S ribosomal protein L21
At3g01550	-1.54	-1.48		-3.30	-5.21	-2.88	triose phosphate/phosphate translocator
At4g35090	-3.20	-2.80	-1.97	-1.74	-4.43	-2.82	catalase 2
At5g64040	-1.87	-2.53	-2.75	-3.26	-3.66	-2.81	photosystem I reaction center
At4g18480	-1.84	-3.15	-1.54	-2.78	-4.57	-2.78	magnesium-chelatase
At2g06520			-1.45	-3.50	-3.35	-2.76	membrane protein
At2g35370	-1.41	-2.05	-1.28	-4.60	-4.47	-2.76	glycine cleavage system H protein 1
At4g34620	-1.65	-2.98	-2.08	-3.78	-3.30	-2.76	ribosomal protein S16 family protein
At4g07310	-1.15	-0.16	-5.43	-2.08	-4.96	-2.75	hypothetical protein
At5g07020	-2.19	-1.74	-1.04	-3.65	-5.13	-2.75	proline-rich family protein
At3g57040	-3.27	-3.39	-1.57			-2.74	two-component responsive regulator
At4g35130	-1.87	-2.94	-2.23	-2.71	-3.94	-2.74	pentatricopeptide (PPR) repeat-containing protein
At3g47070	-0.95	-3.21	-1.50	-3.54	-4.47	-2.73	expressed protein
At1g09340	-2.80	-2.62	-1.20	-4.06	-2.97	-2.73	expressed protein
At3g02730	-0.83	-2.90	-1.80	-3.71	-4.28	-2.70	thioredoxin
At5g51790	-1.68	-1.35	-5.08			-2.70	basic helix-loop-helix (bHLH) family protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At1g48350	-1.38	-2.25	-1.23	-3.93	-4.70	-2.70	ribosomal protein L18 family protein
At2g21710	-2.81	-2.48	-2.16	-2.36	-3.67	-2.69	mitochondrial transcription termination factor-related
At4g24770	-2.03	-1.92	-1.86	-3.56	-4.06	-2.68	31 kDa ribonucleoprotein
At2g46820	-1.97	-2.33	-1.61	-3.40	-4.06	-2.67	expressed protein
At4g39800	-1.28	-3.75	-3.96	-2.14	-2.20	-2.67	inositol-3-phosphate synthase isozyme 1
At1g74670	-2.21	-1.89	-1.80	-4.17	-3.22	-2.66	gibberellin-responsive protein
At5g62840	-1.45	-1.38	-5.11			-2.65	phosphoglycerate
At1g51400	-1.61	-2.28	-2.60	-2.78	-3.95	-2.65	photosystem II 5 kD protein
At1g66100	-2.46	-0.33	-1.17	-4.86	-4.37	-2.64	thionin
At3g26650	-2.30	-1.20	-0.68	-4.09	-4.91	-2.64	GAPA
At3g19800	-2.13	-3.13		-2.66		-2.64	expressed protein
At3g01500	-2.20	-2.52	-1.77	-3.17	-3.41	-2.62	carbonic anhydrase 1
At5g63930	-2.23	-3.42	-1.76	-3.05		-2.61	leucine-rich repeat transmembrane protein kinase
At2g29980	-2.26	-2.58	-0.27	-3.88	-4.03	-2.6	omega-3 fatty acid desaturase
At5g08050	-1.33	-2.50	-1.38	-3.29	-4.45	-2.59	expressed protein
At3g50820	-1.94	-2.93	-0.99	-3.17	-3.89	-2.58	oxygen-evolving enhancer protein
At5g19220	-2.31	-2.33	-0.66	-3.74	-3.84	-2.58	glucose-1-phosphate adenylyltransferase
At3g21055	-1.20	-3.07	-1.64	-3.49	-3.43	-2.57	photosystem II 5 kD protein
At3g51510	-1.33	-1.58	-1.76	-4.07	-4.02	-2.55	expressed protein
At5g14060	-1.14	-1.81	-0.84	-6.41		-2.55	aspartate kinase
At2g05620	-1.42	-3.15	-2.29	-1.56	-4.32	-2.55	expressed protein
At1g65510	-1.45	-1.53	-2.00	-2.63	-5.03	-2.53	expressed protein
At1g05385	-0.96	-1.94	-5.45	-1.74		-2.52	photosystem II 11 kDa protein-related
At1g03630	-1.26	-2.32	-1.03	-3.42	-4.49	-2.5	protochlorophyllide reductase C
At5g11070	-0.98	-3.22	-1.03	-3.20	-4.05	-2.5	expressed protein
At5g40950	-1.56	-2.74	-1.44	-2.74	-3.89	-2.47	50S ribosomal protein L27
At2g29180	-1.98	-2.17	-1.31	-2.88	-4.03	-2.47	expressed protein
At2g24090	-1.74	-1.75	-1.01	-3.43	-4.36	-2.46	ribosomal protein L35 family protein
At1g30380	-1.48	-2.77	-0.60	-4.01	-3.43	-2.46	photosystem I reaction center subunit psaK
At1g52230	-0.74	-2.71	-2.20	-3.82	-2.65	-2.42	photosystem I reaction center subunit VI
At4g17560	-1.39	-2.11	-2.16	-3.57	-2.85	-2.42	ribosomal protein L19 family protein
At1g22630	-2.41	-2.68	-1.24	-3.33		-2.41	expressed protein
At5g64150	-1.61	-1.55	-1.76	-3.12	-4.02	-2.41	methylase family protein
At5g48790			-0.69	-2.41	-4.14	-2.41	expressed protein
At4g21280	-0.87	-1.56	-1.19	-4.30	-4.10	-2.40	oxygen-evolving enhancer protein 3
At3g27050	-2.25	-1.87	-1.92	-1.16	-4.80	-2.40	expressed protein
At1g10470	-2.77	-2.46	-1.33	-2.46	-2.97	-2.40	two-component responsive regulator
At5g26742	-1.20	-1.61	-3.77	-3.00		-2.40	DEAD box RNA helicase
At1g71500	-1.49	-2.13	-1.66	-2.66	-4.05	-2.40	Rieske [2Fe-2S] domain-containing protein
At3g49720	-0.36	-1.55		-4.01	-3.65	-2.39	expressed protein
At1g22020	-2.58	-2.51	-1.27	-1.60	-3.99	-2.39	glycine hydroxymethyltransferase
At3g27160	-1.18	-1.55	-3.47	-2.60	-3.13	-2.38	ribosomal protein S21 family protein
At5g39210	-1.71	-3.54		-1.89		-2.38	expressed protein
At1g04420	-2.03	-1.37	-0.44	-2.51	-5.51	-2.37	aldo/keto reductase family protein
At4g13500	-2.40	-3.02	-1.70			-2.37	expressed protein
At5g66190	-1.41	-1.91	-0.60	-3.69	-4.24	-2.37	ferredoxin NADP(+) reductase
At3g56650	-1.09	-1.34	-0.84	-3.75	-4.83	-2.37	thylakoid lumenal 20 kDa protein
At5g17230	-1.56	-2.72	-1.28	-1.62	-4.66	-2.37	phytoene synthase (PSY)
At5g07200	-2.70	-2.40	-1.50	-2.15	-3.06	-2.36	gibberellin 20-oxidase
At4g04640	-0.43	-2.62	-1.12	-3.29	-4.30	-2.35	ATP synthase gamma chain 1
At5g36120	-1.12	-2.35	-3.58			-2.35	YGGT family protein
At3g56940	-1.70	-2.17	-0.93	-3.17	-3.74	-2.34	dicarboxylate diiron protein
At3g20740	-1.24	-1.07		-4.71		-2.34	fertilization-independent endosperm protein
At4g18740	-1.62	-1.61	-2.40	-2.26	-3.80	-2.34	expressed protein
At1g18060	-3.06	-1.91	-0.73	-3.65		-2.34	expressed protein
At1g29430	-2.08	-1.31	-3.61			-2.33	auxin-responsive family protein
At1g52510	-2.33	-1.83	-0.79	-2.57	-4.15	-2.33	hydrolase, alpha/beta fold family protein
At1g60950	-0.71	-2.61	-1.29	-2.83	-4.17	-2.32	ferredoxin
At2g43030	-1.53		-1.21	-2.91	-3.62	-2.32	ribosomal protein L3 family protein
At2g10940	-1.72	-1.57		-4.29	-3.96	-2.31	protease inhibitor/seed storage/lipid transfer protein
At1g67700	-1.86	-1.70	-1.33	-2.73	-3.85	-2.29	expressed protein
At1g05190	-1.63	-2.55	-0.97	-3.13	-3.15	-2.28	ribosomal protein L6 family protein
At5g24190	-0.65	-2.11	-4.07			-2.28	hypothetical protein
At5g64850	-1.78	-1.77	-0.34	-2.94	-4.57	-2.28	expressed protein
At2g26080	-1.89	-2.16	-0.61	-2.09	-4.62	-2.27	glycine dehydrogenase
At1g66910	-1.67	-0.88	-3.78	-2.03	-3.01	-2.27	protein kinase
At2g43560	-1.87	-2.82	-1.07	-2.87	-2.74	-2.27	immunophilin
At1g29070	-1.49	-1.91	-0.29	-3.71	-3.95	-2.27	ribosomal protein L34 family protein
At3g52320	-2.37	-2.86	-1.58			-2.27	F-box family protein
At5g37360	-0.13	-0.29	-4.74	-3.89		-2.26	expressed protein
At3g56910	-1.61	-1.91	-1.20	-3.21	-3.33	-2.25	expressed protein
At5g65010	-2.56	-1.94	-0.43	-2.26	-4.08	-2.25	asparagine synthetase 2 (ASN2)
At1g20795	-2.84	-1.84	-2.07			-2.25	F-box family protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At2g33450	-1.83	-2.40	-0.77	-3.02	-3.17	-2.24	50S ribosomal protein L28
At4g20360	-0.70	-2.68	-1.67	-2.31	-3.76	-2.22	elongation factor Tu / EF-Tu (TUFA)
At1g68010	-1.10	-0.99	-0.65	-3.71	-4.63	-2.22	glycerate dehydrogenase
At3g55630	-2.31	-3.25		-1.02		-2.19	dihydrofolate synthetase
At2g05070	-1.41	-0.55	-1.88	-2.53	-4.60	-2.19	chlorophyll A-B binding protein
At4g10340	-0.57	-1.44	-1.85	-4.38	-2.66	-2.18	chlorophyll A-B binding protein
At5g02160	-1.53	-1.13	-1.67	-4.19	-2.36	-2.18	expressed protein
At3g29280	-2.94	-0.74	-1.36	-2.57	-3.2	-2.16	expressed protein
At2g21210	-4.25	-1.85	-0.39			-2.16	auxin-responsive protein
At1g56190	-1.87	-2.27	-1.68	-2.26	-2.68	-2.15	phosphoglycerate kinase
At4g01310	-1.12	-2.06	-1.39	-3.29	-2.88	-2.15	ribosomal protein L5 family protein
At1g06680	-0.87	-1.59	-0.96	-3.73	-3.59	-2.15	photosystem II oxygen-evolving complex 23
At1g73655	-1.17	-2.22	-1.31	-2.32	-3.71	-2.15	immunophilin
At1g65230	-2.36	-3.35	-0.73			-2.15	expressed protein
At4g34830	-1.44	-1.55		-3.45		-2.15	pentatricopeptide (PPR) repeat-containing protein
At5g56500	-2.03	-2.48	-1.91			-2.14	chaperonin
At1g64680	-2.78	-2.96	-0.67			-2.14	expressed protein
At1g54780	-1.75	-2.16	-1.16	-2.88	-2.73	-2.13	thylakoid lumen 18.3 kDa protein
At5g66990	-1.45	-0.51	-4.41			-2.12	RWP-RK domain-containing protein
At4g34190	-0.21	-3.21	-0.66	-3.15	-3.34	-2.12	stress enhanced protein 1
At5g66570	-0.82	-1.78	-1.79	-3.20	-2.93	-2.10	oxygen-evolving enhancer protein 1-1
At2g22230	-1.29	-0.75		-2.76	-3.6	-2.10	beta-hydroxyacyl-ACP dehydratase
At1g76160	-1.01	-1.55	-1.42	-3.43	-3.07	-2.10	multi-copper oxidase type I family protein
At1g78630	-1.52	-2.21	-0.20	-3.45	-3.08	-2.09	ribosomal protein L13 family protein
At1g43560	-1.71	-2.36	-1.14	-1.99	-3.26	-2.09	thioredoxin family protein
At5g35490	-1.96	-2.40	-0.03	-2.73	-3.34	-2.09	expressed protein
At3g22210	-1.54	-2.67	-1.29	-2.73	-2.21	-2.09	expressed protein
At1g23310	-1.90	-1.95	-2.03	-2.13	-2.42	-2.08	glutamate:glyoxylate aminotransferase 1
At5g13630	-1.97	-2.12	-1.25	-1.59	-3.48	-2.08	magnesium-chelatase
At3g02690	-1.15	-1.82	-1.05	-4.28		-2.07	integral membrane family protein
At5g59480	-0.47	-1.93	-0.73	-3.43	-3.79	-2.07	haloacid dehalogenase-like
At5g24710	-1.77	-2.99	-1.56	-1.95	-2.08	-2.07	WD-40 repeat family protein
At3g63190	-2.16	-0.83	-0.49	-3.17	-3.69	-2.07	ribosome recycling factor
At5g02110	-1.92	-1.83	-1.87	-2.66		-2.07	cyclin family protein
At1g29420	-1.24	-1.22	-3.71			-2.06	auxin-responsive family protein
At3g62960	-3.27	-1.69	-1.21			-2.06	glutaredoxin family protein
At2g22830	-1.23	-0.02	-4.59	-2.36		-2.05	squalene monooxygenase
At2g26500	-1.29	-2.03	-2.00	-2.09	-2.78	-2.04	cytochrome b6f complex subunit (petM)
At1g01840	-1.26	-0.65	-0.83	-3.78	-3.67	-2.04	expressed protein
At2g23600	-1.88	-2.12	-0.72	-2.80	-2.66	-2.03	hydrolase, alpha/beta fold family protein
At4g28750	-0.86	-0.79	-1.80	-3.97	-2.75	-2.03	photosystem I reaction center subunit IV
At1g58520	-2.13	-3.82	-0.29	-1.87		-2.03	early-responsive to dehydration protein-related
At5g19850	-1.84	-0.98	-1.38	-1.76	-4.13	-2.02	hydrolase, alpha/beta fold family protein
At3g56010	-1.04	-1.70	-2.42	-1.61	-3.34	-2.02	expressed protein
At4g23870	-1.08	-2.94	-1.76		-2.28	-2.01	expressed protein
At1g65500	-2.49	-3.68	-1.52	-0.79	-1.59	-2.01	expressed protein
At4g32260	-1.23	-1.02	-0.63	-3.48	-3.65	-2.00	ATP synthase
At4g37550	-2.07	-1.24	-1.26		-3.41	-2.00	formamidase
At4g03280	-1.38	-1.65	-1.05	-2.55	-3.28	-1.98	cytochrome B6-F complex iron-sulfur subunit
At5g24150	-1.79	-2.32		-1.80		-1.97	squalene monooxygenase
At3g63410	-1.49	-1.49	0.33	-3.29	-3.90	-1.97	chloroplast inner envelope membrane protein
At3g49470	-0.99	-0.88		-2.56	-3.43	-1.97	nascent polypeptide-associated complex
At1g26700	-2.80	-2.42	0.69	-3.18	-2.12	-1.97	seven transmembrane MLO family protein
At2g32180	-1.84	-2.59	-0.79		-2.64	-1.96	expressed protein
At5g38520	-1.92	-2.84	0.37	-3.44		-1.96	hydrolase, alpha/beta fold family protein
At5g06290	-1.81	-1.63	-1.23		-3.16	-1.95	2-cys peroxiredoxin
At4g05340	-0.50		-1.70		-3.66	-1.95	hypothetical protein
At1g29910	-1.20	-0.31	-2.20	-4.48	-1.55	-1.95	chlorophyll A-B binding protein 2
At5g24314	-1.81	-1.97	-0.27	-3.74		-1.95	expressed protein
At2g03750	-1.53	-2.11	-2.19			-1.94	sulfotransferase family protein
At4g30620	-1.03	-1.46	-1.99	-2.16	-3.06	-1.94	expressed protein
At3g11170	-1.76	-2.23	-0.67	-2.95	-2.07	-1.93	omega-3 fatty acid desaturase
At5g54270	-0.29	-0.65	-1.73	-4.34	-2.66	-1.93	chlorophyll A-B binding protein
At5g30510	-1.33	-2.17	-1.23	-1.97	-2.93	-1.93	30S ribosomal protein S1
At5g41570		-0.64	-2.19	-2.95		-1.93	WRKY family transcription factor
At2g28180	-1.19	-0.97	-3.61			-1.93	cation/hydrogen exchanger
At1g62750	-1.98	-2.51	-0.10	-1.69	-3.28	-1.91	elongation factor Tu family protein
At2g21490	-2.35	-2.63	-0.98	-0.38	-3.2	-1.91	dehydrin family protein
At2g25080	-1.77	-0.82	-1.43	-2.64	-2.87	-1.91	phospholipid hydroperoxide glutathione peroxidase
At2g42600	-1.35	-1.57	-0.50	-2.52	-3.59	-1.91	phosphoenolpyruvate carboxylase
At2g39840	-2.37	-3.43	-1.77	-0.81	-1.12	-1.90	Ser/Thr phosphatase
At1g02320	-3.63	-1.55	-0.52			-1.90	hypothetical protein
At3g62410	-1.54	-3.04	-0.01	-2.27	-2.61	-1.89	CP12 domain-containing protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At2g30790	-0.87	-1.35	-1.73	-2.34	-3.12	-1.88	photosystem II oxygen-evolving complex 23
At4g27700	-1.24	-1.29	-1.49	-3.27	-2.10	-1.88	rhodanese-like domain-containing protein
At4g25100	-0.94	-1.70	-2.30	-2.22	-2.21	-1.87	superoxide dismutase
At1g04240	-1.29	-0.76	-0.90	-3.18	-3.22	-1.87	auxin-responsive protein
At2g23000	-1.88	-0.99	-1.53	-0.44	-4.51	-1.87	serine carboxypeptidase S10 family protein
At5g12860	-1.79	-2.02		-1.79		-1.87	oxoglutarate/malate translocator
At2g28605	-2.25	-1.61	-1.55	-2.05		-1.86	expressed protein
At4g38970	-1.02	-1.63	-0.16	-3.16	-3.36	-1.86	fructose-bisphosphate aldolase
At4g22890	-0.76	-2.23	-1.05	-2.37	-2.88	-1.86	expressed protein
At1g27950	-0.64	-1.67	-0.18	-3.11	-3.69	-1.86	lipid transfer protein-related
At1g67150	-0.84	-2.78	-1.60	-2.20		-1.86	hypothetical protein
At4g02920	-1.48	-2.00	-0.20	-2.06	-3.53	-1.85	expressed protein
At3g44890	-1.24	-2.05	-0.71	-2.63	-2.63	-1.85	50S ribosomal protein L9
At2g38140	-0.90	-1.72	-0.98	-2.86	-2.78	-1.85	chloroplast 30S ribosomal protein S31
At1g05850	-0.74	-1.32	-1.39	-3.03	-2.75	-1.85	chitinase-like protein 1 (CTL1)
At4g10470	-1.74	-1.95	-0.15	-3.54		-1.85	expressed protein
At4g31800	-0.56	-0.77	-0.79	-3.57	-3.54	-1.85	WRKY family transcription factor
At4g21130	-2.00	-2.76	-0.76			-1.84	transducin family protein
At4g32760	-1.98	-2.27	0.23	-2.18	-2.98	-1.83	VHS domain-containing protein
At1g63960	-2.46	-1.42	-0.28	-2.17	-2.84	-1.83	hypothetical protein
At2g02590	-1.67	-2.28	-1.46	-1.91		-1.83	expressed protein
At2g15290	-1.03	-0.64	-3.81	-1.54	-2.13	-1.83	expressed protein
At3g26570	-2.09	-2.87	-0.52			-1.83	phosphate transporter family protein
At4g05470	-0.68	-0.08	-4.72			-1.83	F-box family protein (FBL21)
At5g14740	-2.10	-1.61	-0.02	-2.42	-2.99	-1.82	carbonic anhydrase 2
At5g45950	-1.50	-3.14	-1.30	-1.35		-1.82	GDSL-motif lipase/hydrolase family protein
At1g13380	-0.60	-0.88	-1.00	-3.32	-3.31	-1.82	expressed protein
At4g23260	-2.04	-1.95	-2.18	-1.02	-1.89	-1.82	protein kinase family protein
At1g70410	-0.86	-1.73	-0.29	-3.09	-3.09	-1.81	carbonic anhydrase
At2g36990	-1.03	-1.56		-1.49	-3.15	-1.81	RNA polymerase sigma subunit SigF (sigF)
At2g46830	-3.17	-1.95	-1.63	-0.44	-1.83	-1.81	myb-related transcription factor (CCA1)
At3g01440	-0.63	-1.83	-0.55	-2.28	-3.73	-1.8	oxygen evolving enhancer 3
At2g23590	-2.45	-2.28	-0.66			-1.8	hydrolase, alpha/beta fold family protein
At5g16200	0.16	-1.60	-1.83	-3.91		-1.79	50S ribosomal protein-related
At5g44650	-0.72	-2.50	-1.36	-2.57		-1.79	expressed protein
At5g59800	-1.60	-1.27	-1.54	-2.73		-1.78	methyl-CpG-binding domain-containing protein
At1g65080	-1.66	-2.44	-1.22			-1.78	OXA1 family protein
At1g22430	-1.90	-3.40	-0.03			-1.77	alcohol dehydrogenase
At5g46110	-0.63	-1.46	-1.18	-2.69	-2.88	-1.77	phosphate/triose-phosphate translocator
At4g32980	-0.68	-1.62	-3.00			-1.77	homeobox protein
At5g26780	-1.95	-1.82	-0.69	-1.15	-3.23	-1.77	glycine hydroxymethyltransferase
At1g24020	-1.84	-2.50	-0.96			-1.77	Bet v I allergen family protein
At3g52230	-0.73	-2.30		-1.44	-2.59	-1.76	expressed protein
At4g29905	-1.98	-3.45	-0.77	-0.85		-1.76	expressed protein
At5g17870	-1.08	-1.77	-0.73	-2.56	-2.62	-1.75	plastid-specific ribosomal protein-related
At5g37260	-0.68	-4.01	-0.69	-0.95	-2.43	-1.75	myb family transcription factor
At5g59870	-1.61	-3.30		-1.08	-1.00	-1.75	histone H2A
At3g50530	-2.51	-2.32	-0.40			-1.75	calcium-dependent protein kinase
At4g18810	-0.69	-1.84	-0.29	-3.40	-2.50	-1.74	expressed protein
At4g21870	-1.77	-0.98	-2.48			-1.74	Heat shock protein 18.2
At1g55670	-1.50	-0.95	-1.03	-3.06	-2.16	-1.74	photosystem I reaction center subunit V
At5g39270	-1.92	-1.82	-1.44			-1.73	expansin (EXP22)
At5g48545			-0.77	-1.93	-2.47	-1.72	histidine triad family protein
At3g27690	-1.12	-0.47	-1.18	-2.67	-3.16	-1.72	chlorophyll A-B binding protein
At5g14260	-0.74	-1.21	-3.08	-0.91	-2.65	-1.72	SET domain-containing protein
At1g17050	-1.84	-2.48		-0.83		-1.72	geranyl diphosphate synthase
At4g17090	-0.50	-2.74	-0.57	-2.32	-2.42	-1.71	beta-amylase (CT-BMY)
At4g35100	-0.64	-1.87	-1.41	-1.35	-3.27	-1.71	plasma membrane intrinsic protein
At5g02840	-1.10	-1.18	-1.27	-1.26	-3.7	-1.70	myb family transcription factor
At4g12440	-1.26	-2.43	-1.39	-1.68	-1.74	-1.70	adenine phosphoribosyltransferase
At5g19940	-0.80	-0.90	-1.34	-2.20	-3.22	-1.69	plastid-lipid associated protein PAP-related
At2g04700	-0.57	-1.88	-2.16	-2.04	-1.76	-1.68	ferredoxin thioredoxin reductase
At1g12580	-1.07	-2.76	-0.63	-1.36	-2.57	-1.68	protein kinase family protein
At1g55490	-1.17	-1.41	-0.47	-2.78	-2.55	-1.68	RuBisCO subunit binding-protein beta subunit
At5g15850	-2.09	-2.56	-0.57	-0.47	-2.68	-1.67	zinc finger protein CONSTANS-LIKE 1
At4g23240	-1.15	-0.91	-0.34	-3.56	-2.4	-1.67	protein kinase family protein
At2g47400	-0.52	-2.12	-1.68	-1.59	-2.45	-1.67	CP12 domain-containing protein
At2g37220	-0.96	-1.90	-0.20	-2.56	-2.71	-1.67	29 kDa ribonucleoprotein
At3g24320	-1.49	-0.57	-2.94			-1.66	DNA mismatch repair MutS family (MSH1)
At4g39330	-0.73	-3.24	-1.01			-1.66	mannitol dehydrogenase
At2g24270	-0.14	-1.17	-1.18	-2.91	-2.90	-1.66	NP-GAPDH
At5g11450	-2.35	-3.09	0.74	-1.95		-1.66	oxygen-evolving complex-related
At2g33180	-1.22	-1.42	0.08	-2.05	-3.70	-1.66	expressed protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At1g73310	-1.30	-1.76	-2.59	-0.38	-2.27	-1.66	serine carboxypeptidase S10 family protein
At5g26000	-1.04	-2.20	-1.60	-1.99	-1.47	-1.66	glycosyl hydrolase family 1 protein
At5g15350	-1.59	-0.79	-0.87	-1.70	-3.32	-1.65	plastocyanin-like domain-containing protein
At2g30420	-1.95	-1.33	-1.65			-1.65	myb family transcription factor
At2g28440	-1.62	-1.46	-0.82	-1.65	-2.69	-1.65	proline-rich family protein
At3g01480	-1.86	-1.21	-0.12	-2.87	-2.11	-1.63	peptidyl-prolyl cis-trans isomerase
At3g09640	-1.30	-1.16	-0.57	-2.97	-2.10	-1.62	L-ascorbate peroxidase 1b (APX1b)
At3g21960	-1.63	-1.94	-0.86	-1.68	-1.99	-1.62	receptor-like protein kinase-related
At1g53300	-1.55	-0.66	-0.96	-2.19	-2.72	-1.62	thioredoxin family protein
At1g65320	-1.35	-1.80	-0.40	-1.92	-2.57	-1.61	CBS domain-containing protein
At1g64510	-1.00	-1.68	-0.80	-2.28	-2.27	-1.61	ribosomal protein S6 family protein
At1g67310	-1.74	-3.27	0.26	-1.67		-1.61	calmodulin-binding protein
At2g41470	-1.05	-1.29	-0.91	-2.36	-2.42	-1.60	embryo-specific protein-related
At2g23610	-0.97	-1.38	-0.42		-3.65	-1.60	esterase
At1g10960	-0.20	-1.49	-1.20	-2.15	-2.98	-1.60	ferredoxin, chloroplast
At4g32190	-0.52	-1.96	-0.48	-1.61	-3.43	-1.60	centromeric protein-related
At1g45474	-1.63	-1.87	-0.34	-1.75	-2.38	-1.59	chlorophyll A-B binding protein
At4g37230	-0.98	-2.17	-0.56	-1.67	-2.58	-1.59	oxygen-evolving enhancer protein
At2g35130	-0.61	-2.62		-1.55		-1.59	pentatricopeptide (PPR) repeat-containing protein
At1g77760	-2.83	-0.77	-0.91	-2.24	-1.16	-1.58	nitrate reductase 1 (NR1)
At1g70090	-0.75	-1.58	-0.75	-1.23	-3.61	-1.58	glycosyl transferase family 8 protein
At1g12560	-1.68	-1.66	-1.39			-1.58	expansin (EXP7)
At5g19370	-0.39	-1.71	-0.65	-2.48	-2.66	-1.58	rhodanese-like domain-containing protein
At1g02010	-1.78	-1.75	-1.20			-1.58	cytokinesis-related Sec1 protein
At5g34887	-1.53	-1.54	-1.66			-1.58	expressed protein
At3g26070	-0.73	-1.34	-0.76	-2.44	-2.59	-1.57	plastid-lipid associated protein PAP
At5g57920	-1.40	-1.60	-0.17	-2.49	-2.19	-1.57	plastocyanin-like domain-containing protein
At4g27657	0.07	-2.57	-1.48	-2.54	-1.32	-1.57	expressed protein
At1g11655	-1.66	-1.07	-1.77	-1.56	-1.77	-1.56	expressed protein
At4g02680	-1.75	-2.30	-1.13	-0.84	-1.81	-1.56	tetratricopeptide repeat
At2g30570	-0.15	-1.15	-1.64	-2.43	-2.45	-1.56	photosystem II reaction center W
At1g10370	-0.49	-1.64	-0.91	-2.84	-1.92	-1.56	glutathione S-transferase
At1g32220	-1.55	-1.32	-0.53	-2.85		-1.56	expressed protein
At3g21465	-2.23	-2.14	-0.79	-0.29	-2.33	-1.56	expressed protein
At5g20630	-1.59	-3.27	-0.58	-1.22	-1.08	-1.55	germin-like protein (GER3)
At2g25480	-1.58	-1.57	-0.65	-1.76	-2.16	-1.54	expressed protein
At4g28025	-1.29	-1.76		-0.49	-2.62	-1.54	expressed protein
At3g14130		-1.23	-0.60	-2.07	-2.22	-1.53	(S)-2-hydroxy-acid oxidase
At1g77490	-0.81	-1.09	-0.76	-2.71	-2.27	-1.53	L-ascorbate peroxidase
At4g09400	-0.79	-0.70	-1.94	-2.60	-1.61	-1.53	hypothetical protein
At4g13070	-1.30	-2.19	-1.09			-1.53	group II intron splicing factor CRS1-related
At5g19190	-1.95	-1.42	-0.83	-1.30	-2.14	-1.53	expressed protein
At5g27780	-1.64	-1.67	-1.25			-1.52	auxin-responsive family protein
At4g31780	-0.85	-0.74	-0.27	-2.69	-3.04	-1.52	monogalactosyldiacylglycerol synthase
At4g09650	-0.60	-2.43	-0.72	-1.53	-2.30	-1.51	ATP synthase delta chain
At4g16810	-1.13	-2.63	-0.76			-1.51	expressed protein
At5g06090	-2.08	-1.07	-1.37			-1.51	phospholipid/glycerol acyltransferase
At2g41680	-1.81	-2.27	-0.70	-1.25		-1.50	thioredoxin reductase
At2g33255	-0.60	-1.44	-0.80		-3.18	-1.50	haloacid dehalogenase-like
At1g72630	-1.19	-0.82	-0.91	-1.56	-3.04	-1.50	expressed protein
At1g52280	-0.69	-1.24	-3.21	-1.57	-0.77	-1.50	Ras-related GTP-binding protein
At4g01150	-0.87	-1.50	-0.14	-2.74	-2.23	-1.50	expressed protein
At2g05790	-2.46	-1.22	-0.31	-1.98		-1.49	glycosyl hydrolase family 17 protein
At4g27654	0.21	-2.98	-1.23		-1.97	-1.49	expressed protein
At3g12700	-0.14	-0.92	-2.69	-2.22		-1.49	aspartyl protease family protein
At1g32900	-1.37	-1.09	-0.27	-1.95	-2.80	-1.49	starch synthase
At2g21385	-2.28	-1.11	-0.79		-1.80	-1.49	expressed protein
At5g53780	-0.24		-2.05	-2.18		-1.49	hypothetical protein
At5g16540	-1.09	-1.74	-1.00	-1.30	-2.29	-1.48	zinc finger (CCCH-type) family protein
At5g01530	-0.32	-0.91	-1.34	-2.73	-2.10	-1.48	chlorophyll A-B binding protein
At3g07690	-2.12	-2.45	-0.37	-0.98		-1.48	glycerol-3-phosphate dehydrogenase
At1g79840	-2.56	-2.28	-0.97	-0.74	-0.84	-1.48	homeobox-leucine zipper protein 10
At2g41820	-1.62	-1.97	-0.84			-1.47	leucine-rich repeat transmembrane protein kinase
At3g05490	-0.56	-1.21	-0.55	-1.71	-3.34	-1.47	rapid alkalinization factor (RALF) family protein
At2g27630	-0.74	-0.39	-1.44	-1.60	-3.19	-1.47	ubiquitin carboxyl-terminal hydrolase-related
At1g50250	-0.81	-1.89	-1.04	-0.94	-2.67	-1.47	cell division protein ftsH homolog 1
At2g24020	-0.94	-1.71	-0.88	-2.09	-1.72	-1.47	expressed protein
At3g57120	-0.43	-2.09	-0.65	-2.00	-2.18	-1.47	protein kinase family protein
At5g23740	-0.61	-0.46	-2.79	-2.31	-1.17	-1.47	40S ribosomal protein S11
At5g18140	-1.23	-0.89	-0.70	-1.22	-3.29	-1.47	DNAJ heat shock N-terminal
At3g02830	-1.02	-2.93	0.38	-2.30		-1.47	zinc finger (CCCH-type) family protein
At2g42975	-1.77	-1.75	-0.85	-1.48		-1.46	expressed protein
At1g32990	-1.46	-0.97	-0.37	-2.44	-2.07	-1.46	ribosomal protein L11 family protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At4g24930	-1.66	-2.12	0.15	-1.88	-1.79	-1.46	thylakoid lumenal 17.9 kDa protein
At1g52590	-2.10	-1.40	-0.94	-1.20	-1.66	-1.46	expressed protein
At5g10150	-1.20	-0.58	-2.60			-1.46	expressed protein
At3g04920	-0.99	-2.34	-0.28	-1.81	-1.86	-1.46	40S ribosomal protein S24
At4g26350	-2.21		0.07	-2.23		-1.46	F-box family protein contains F-box domain
At3g48360	-2.08	-1.45	-2.68	-1.54	0.48	-1.45	speckle-type POZ protein-related
At1g44446	-1.66	-2.41	-0.07	-0.74	-2.38	-1.45	chlorophyll a oxygenase (CAO)
At2g34460	-1.15	-2.52	-0.04	-1.79	-1.75	-1.45	flavin reductase-related
At1g23740	-0.93	-1.76	-0.59	-1.46	-2.49	-1.45	oxidoreductase,n
At1g49220	-1.77	-1.54	-1.02			-1.44	zinc finger (C3HC4-type RING finger) family protein
At3g44910	-2.40	-0.80	-0.66		-1.91	-1.44	cation/hydrogen exchanger
At1g76450	-0.06	-1.73	-0.61	-1.94	-2.86	-1.44	oxygen-evolving complex-related
At5g17300	-0.98	-2.08	-1.25	-0.73	-2.16	-1.44	myb family transcription factor
At3g56370	-0.61	-2.62	-1.08			-1.43	leucine-rich repeat transmembrane protein kinase
At1g16170	-2.04	-0.25		-1.52	-1.92	-1.43	expressed protein
At2g36835	-0.81	-2.04	-0.40	-1.19	-2.69	-1.43	expressed protein
At1g68590	-0.91	-2.05	-0.68	-1.55	-1.93	-1.42	plastid-specific 30S ribosomal protein 3
At1g45010	-1.29	-2.43	-1.14	-1.57	-0.69	-1.42	expressed protein
At3g11550	-2.70	-2.33	0.18	-0.84		-1.42	integral membrane family protein
At2g21170	-0.61	-1.37	-0.27	-2.29	-2.56	-1.42	triosephosphate isomerase
At3g54210	-0.35	-1.23	-0.75	-1.97	-2.78	-1.42	ribosomal protein L17 family protein
At2g21220	-1.73	-1.33	-1.18			-1.41	auxin-responsive protein
At5g20140	-1.34	-2.33	-0.56			-1.41	SOUL heme-binding family protein
At1g44970	-2.08	-1.40	0.58		-2.71	-1.40	peroxidase
At3g19030	-0.38	-0.94	-1.33	-2.28	-2.07	-1.40	expressed protein
At2g20930	-1.25	-1.79	-1.04	-1.53	-1.37	-1.40	expressed protein
At3g55800	-0.69	-1.73	-0.64	-1.55	-2.36	-1.39	sedoheptulose-1,7-bisphosphatase
At3g62270	-1.64	-1.32	-1.01	-1.32	-1.66	-1.39	anion exchange family protein
At5g15660	-2.09	-0.93	-1.13			-1.38	F-box family protein
At3g29240	-0.72	-1.07	-1.14	-0.67	-3.33	-1.38	expressed protein
At3g25660	-1.71	-1.83	-0.61			-1.38	glutamyl-tRNA(Gln) amidotransferase
At1g18420	-0.69	-0.13	-1.75		-2.95	-1.38	expressed protein
At3g60890	-1.69	-0.27	-0.41	-1.97	-2.57	-1.38	hypothetical protein
At2g28800	-1.06	-0.92	-1.97	-1.28	-1.63	-1.37	chloroplast membrane protein
At5g39530	-0.59	-1.46	-0.95	-1.99	-1.84	-1.37	expressed protein
At5g66530	-1.30	-1.14	-0.30	-2.36	-1.73	-1.37	aldose 1-epimerase family protein
At2g30540	-2.93	-1.81	0.64			-1.36	glutaredoxin family protein
At2g39670	-0.67	-1.36	-0.31	-2.79	-1.68	-1.36	radical SAM domain-containing protein
At2g17695	-1.05	-1.71	-0.87	-1.81		-1.36	expressed protein
At4g24780	-1.28	-2.13	-1.16	-1.09	-1.13	-1.36	pectate lyase family protein
At4g36070	-0.61	-2.33	-1.14			-1.36	calcium-dependent protein kinase
At5g45930	-1.48	-1.97	-0.83	0.10	-2.61	-1.36	magnesium-chelatase
At3g60580	-1.16	-1.86	-1.05			-1.36	zinc finger (C2H2 type) family protein
At2g25605	-1.28	-0.90	-0.66	-1.54	-2.40	-1.35	expressed protein
At3g06035	-0.57	-1.62	-0.42		-2.80	-1.35	expressed protein
At1g52220	-0.59	-0.99	-1.15	-2.64	-1.39	-1.35	expressed protein
At4g00030	-0.64	-0.94		-1.20	-2.62	-1.35	plastid-lipid associated protein PAP
At1g29440	-1.48	-1.22	-1.34			-1.35	auxin-responsive family protein
At5g54240	-1.44	-1.78	-0.96	-0.89	-1.67	-1.35	expressed protein
At4g04350	-0.41	-1.14		-2.50		-1.35	leucyl-tRNA synthetase
At2g38730	-1.94	-2.71	-1.14	-0.40	-0.54	-1.35	peptidyl-prolyl cis-trans isomerase
At5g08650	-1.07	-0.65		-1.49	-2.15	-1.34	GTP-binding protein LepA
At1g56120	-1.93	-1.65	-0.44			-1.34	leucine-rich repeat family protein
At4g17470	-1.88	-0.69	-1.45			-1.34	palmitoyl protein thioesterase family protein
At1g16910	-2.56	-1.91	-1.42	-0.19	-0.61	-1.34	hypothetical protein
At3g21000	-1.17	-1.57	-2.03	-0.11	-1.82	-1.34	expressed protein
At1g29700	-1.14	-1.76	-0.26		-2.18	-1.33	expressed protein
At5g55210	-0.19	-1.73	-1.48	-0.60	-2.65	-1.33	expressed protein
At1g16080	-1.18	-1.11	0.13	-2.17	-2.32	-1.33	expressed protein
At3g05410	-0.82	-0.94	-1.01	-2.54		-1.33	expressed protein
At2g25510	0.24	-1.88	-0.75	-2.53	-1.67	-1.32	expressed protein
At3g05000	-1.90		-0.59	-1.94	-0.85	-1.32	transport protein particle (TRAPP) component
At2g26340	-0.18	-1.68		-0.98	-2.43	-1.32	expressed protein
At5g09660	-0.59	-2.29	0.07	-1.82	-1.94	-1.32	malate dehydrogenase
At1g08380	-0.80	-0.82	-0.74	-3.22	-1.00	-1.32	expressed protein
At3g48040	-1.08	-2.12	-0.94		-1.13	-1.32	Rac-like GTP-binding protein (ARAC8)
At4g14060	-2.59	-0.57	-0.79			-1.32	major latex protein-related
At5g36150	-0.77	-2.58	-0.11	-1.49	-1.62	-1.31	pentacyclic triterpene synthase
At5g57100	-1.87	-1.35	-0.73	-1.31		-1.31	transporter-related
At2g22920	-1.24	-1.21	-2.01	-0.33	-1.77	-1.31	serine carboxypeptidase S10 family protein
At3g12230	-2.47	-2.25	-1.20	-0.13	-0.50	-1.31	serine carboxypeptidase S10 family protein
At1g67390	-0.73	-2.60	-0.60			-1.31	F-box family protein
At5g65730	-0.94	-0.75	-1.67	-1.40	-1.79	-1.31	xyloglucan:xyloglucosyl transferase

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At4g25515	-1.41	-1.10	-1.40			-1.31	transcriptional co-regulator family protein
At5g58120	-1.46	-0.89	-0.26	-2.61		-1.31	disease resistance protein
At3g04740	-1.62	-2.17	-0.54	-0.88		-1.31	expressed protein
At5g62570	-0.37	-0.77	-0.82	-2.49	-2.07	-1.30	calmodulin-binding protein
At5g57170	-1.01	-1.51	-0.27	-2.43		-1.30	MIF family protein
At2g24740	-0.42	-0.95		-1.55	-2.31	-1.30	SET domain-containing protein (SUVH8)
At4g37780	-1.68	-1.16	-0.51	-0.69	-2.47	-1.30	myb family transcription factor (MYB87)
At2g04790	-1.01	-1.48	-0.05	-2.64		-1.30	expressed protein
At5g48655	-0.72	-0.52	-0.37	-2.26	-2.61	-1.30	zinc finger (C3HC4-type RING finger) family protein
At1g73270	-1.61	-1.40	-1.41	-0.16	-1.91	-1.29	serine carboxypeptidase S10 family protein
At3g48320	-0.41	-2.35	-1.12			-1.29	cytochrome P450 71A21, putative (CYP71A21)
At2g22990	-1.72	-1.28	-0.99	-0.66	-1.79	-1.29	sinapoylglucose:malate sinapoyltransferase (SNG1)
At5g03080	-0.94	-1.44	-0.36	-1.28	-2.41	-1.29	phosphatidic acid phosphatase-related
At2g28950	-1.40	-2.05	0.04	-1.47	-1.55	-1.29	expansin (EXP6)
At1g52560	-1.00	-0.76		-2.09		-1.29	26.5 kDa class I small heat shock protein-like
At5g37550	-2.08	-1.07	-0.71			-1.28	expressed protein
At3g60210	-0.53	-1.45	-1.01	-1.4	-2.02	-1.28	chloroplast chaperonin 10
At1g70680	-1.54	-1.33	-0.97	-1.38	-1.17	-1.28	calcosin-related family protein
At5g45430	-0.28	-1.30	-0.53	-1.4	-2.90	-1.28	protein kinase
At1g27400	-0.71	-0.97	-1.04	-1.94	-1.72	-1.28	60S ribosomal protein L17
At1g76100	-0.78	-1.38	-0.84	-1.44	-1.94	-1.27	plastocyanin
At5g17400	-2.26	-1.39	-1.06	-0.77	-0.90	-1.27	ADP, ATP carrier protein
At2g33800	-0.51	-1.92	-0.48	-1.94	-1.51	-1.27	ribosomal protein S5 family protein
At3g48010	-1.16	-2.00	-0.66			-1.27	cyclic nucleotide-regulated ion channel
At5g46560	-1.00	-1.49	-1.39	-0.84	-1.62	-1.27	expressed protein
At1g64770	-1.12	-1.71	-0.51	-1.01	-1.98	-1.27	expressed protein
At2g23910	-0.73	-1.92	-1.14			-1.26	cinnamoyl-CoA reductase-related
At4g10770	-1.69	-1.20			-0.90	-1.26	oligopeptide transporter OPT family protein
At4g31290	-0.24	-0.95	-1.47	-1.76	-1.88	-1.26	ChaC-like family protein
At1g77090	-1.18	-1.82	-0.99	-1.05		-1.26	thylakoid lumenal 29.8 kDa protein
At4g24640	-0.81	-1.08	-1.88			-1.26	invertase/pectin methylesterase inhibitor family
At2g42260	-1.53	-1.53	-0.71			-1.26	expressed protein
At5g54290	0.07	-0.99	-0.99	-1.84	-2.52	-1.25	cytochrome c biogenesis protein
At3g54660	-1.38	-1.99	-0.28	-0.47	-2.16	-1.25	gluthatione reductase
At2g25710	-1.27	-1.00	-0.02	-1.89	-2.08	-1.25	holocarboxylase synthetase 1
At1g65260	-0.52	-1.81	-1.14	-1.4	-1.38	-1.25	PspA/IM30 family protein
At5g52570	-0.73	-1.86	0.16	-2.28	-1.53	-1.25	beta-carotene hydroxylase
At5g42070	-0.27	-0.96	-1.21	-1.47	-2.34	-1.25	expressed protein
At2g28190	-1.30	-1.48	-0.75	-1.26	-1.45	-1.25	superoxide dismutase
At1g15180	-1.35	-0.51	-0.09	-1.62	-2.66	-1.25	MATE efflux family protein
At4g16155	-1.17	-1.37	-0.64	-1.54	-1.50	-1.24	dihydrolipoamide dehydrogenase 2
At1g31330	-0.38	-0.66	-0.85	-2.57	-1.76	-1.24	photosystem I reaction center subunit III
At1g20470	-1.11	-1.20	-0.50	-2.15		-1.24	auxin-responsive family protein
At2g24395	-1.16	-1.42	0.28	-1.53	-2.36	-1.24	chaperone protein dnaJ-related
At2g41120	-0.65	-0.98	-0.95	-0.97	-2.65	-1.24	expressed protein
At2g22980	-1.80	-1.44	-1.76	0.06		-1.24	serine carboxypeptidase S10 family protein
At3g60810	-0.33	-1.15	-1.67	-1.50	-1.52	-1.23	expressed protein
At1g80140	-1.18	-2.37	-0.97	-0.39		-1.23	glycoside hydrolase family 28 protein
At5g58300	-0.69	-1.10	-1.41	-1.48	-1.46	-1.23	leucine-rich repeat transmembrane protein kinase
At4g29060	-0.81	-2.36	-1.48	0.03	-1.52	-1.23	elongation factor Ts family protein
At3g08940	-0.33	-0.52	-1.04	-2.29	-1.97	-1.23	chlorophyll A-B binding protein
At1g19510	-1.73	-1.92	-0.04			-1.23	myb family transcription factor
At1g52530	-2.03	-0.32		-0.88	-1.67	-1.23	expressed protein
At3g61100	-0.66	-1.11	-0.50	-2.20	-1.64	-1.22	expressed protein
At1g28410	-1.40	-1.20	-1.07			-1.22	expressed protein
At5g16650	-1.83	-1.02	-0.50	-1.54		-1.22	DNAJ heat shock
At5g22660	-0.59	-1.47	-1.09	-1.01	-1.95	-1.22	F-box family protein
At5g23970	-1.17	-0.76	-0.15	-1.63	-2.40	-1.22	transferase family protein
At1g34000	-0.53	-0.89	-0.68	-1.67	-2.34	-1.22	light stress-responsive one-helix protein
At3g27830	-1.12	-0.94	-0.33	-1.50	-2.22	-1.22	50S ribosomal protein L12-1
At4g34760	-1.36	-1.06	-0.78	-1.27	-1.64	-1.22	auxin-responsive family protein
At1g54500	-0.85	-1.19		-2.15	-1.90	-1.22	rubredoxin family protein
At4g25110	-1.06		-0.57	-0.78	-2.46	-1.22	latex-abundant family protein (AMC2)
At3g48420	-1.25	-0.92	-0.17	-2.23	-1.52	-1.22	haloacid dehalogenase-like
At2g26900	-0.47	-0.27	-1.08	-1.90	-2.35	-1.22	bile acid:sodium symporter family protein
At4g30280	-0.01	-0.79	-1.55	-2.13	-1.60	-1.22	xyloglucan:xyloglucosyl transferase
At1g61740	-0.48	-1.66	-1.85	-1.35	-0.73	-1.21	expressed protein
At1g07770	-0.44	-1.21	-1.62	-1.65	-1.13	-1.21	40S ribosomal protein S15A
At3g14670	-1.57	-1.76	-0.31			-1.21	hypothetical protein
At2g32350	-1.30	-0.20	-0.61	-1.98	-1.96	-1.21	ubiquitin family protein
At1g52870	-0.97	-0.91	-0.61	-1.65	-1.92	-1.21	peroxisomal membrane protein-related
At5g55540	-1.14	-1.31	-1.17			-1.21	expressed protein
At4g01730	-1.72	-1.22	-0.67			-1.21	zinc finger (DHHC type) family protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At2g42110	-0.13	-0.86	-0.80	-2.02	-2.22	-1.21	expressed protein
At5g23790	-1.20	-0.11	-1.54	-1.26	-1.89	-1.20	galactinol synthase
At2g20890	-0.66	-1.17	-0.69	-1.54	-1.94	-1.20	expressed protein
At1g06690	-1.28	-1.86	-1.18	-0.48		-1.20	aldo/keto reductase family protein
At4g21210	-1.00	-1.04	-0.58	-2.17		-1.20	expressed protein
At3g60750	-0.89	-1.63	-1.01	-0.72	-1.74	-1.20	transketolase
At4g15545	-0.77	-0.96	-0.89	-1.45	-1.91	-1.20	expressed protein
At1g48300	-0.50	-2.07	-1.21	-0.42	-1.78	-1.20	expressed protein
At1g11860	-0.33	-2.03	-0.60	-1.98	-1.04	-1.20	aminomethyltransferase
At5g35960	-1.22	-1.60	-0.76			-1.19	protein kinase
At1g74850	-2.04	-1.76	-1.02	-0.38	-0.76	-1.19	pentatricopeptide (PPR) repeat-containing protein
At3g03630	-1.40		-1.27	-0.90		-1.19	cysteine synthase
At1g69530	-2.40	-1.46	-0.74	-0.57	-0.77	-1.19	expansin (EXP1)
At2g43750	-1.11	-0.78	-0.02	-1.84	-2.19	-1.19	cysteine synthase
At5g54380	-1.45	-0.90	-0.03	-1.49	-2.06	-1.19	protein kinase family protein
At5g25980	-0.36	-1.32	-1.89	-1.63	-0.74	-1.19	glycosyl hydrolase family 1 protein
At1g17910	-0.91	-0.24	-2.39			-1.18	wall-associated kinase
At1g43850	-0.11	-2.34	-1.08			-1.18	SEUSS transcriptional co-regulator
At4g38860	-2.04	-0.98	-0.52			-1.18	auxin-responsive protein
At4g09350	-0.71	-1.23	-0.22	-1.64	-2.08	-1.18	DNAJ heat shock
At3g25850	-1.14	-0.42	-0.81	-1.94	-1.57	-1.18	DC1 domain-containing protein
At1g73780	-1.82	-0.84	-0.86			-1.18	protease inhibitor/seed storage/lipid transfer protein
At4g37760	-1.69	-1.02		-0.53	-1.45	-1.17	squalene monooxygenase
At3g24230	-0.60		-0.23		-2.68	-1.17	pectate lyase family protein
At5g54600	-0.57	-1.13	-0.43	-1.45	-2.28	-1.17	50S ribosomal protein L24
At4g13930	-0.29	-2.45	-0.62	-1.64	-0.86	-1.17	glycine hydroxymethyltransferase
At1g80380	-1.28	-1.27	-0.48	-1.31	-1.50	-1.17	phosphoribulokinase/uridine kinase-related
At1g67480	-0.02	-2.23	-0.44	-1.30	-1.86	-1.17	kelch repeat-containing F-box family protein
At1g44830	-1.71	-0.74	-1.07			-1.17	AP2 domain-containing transcription factor
At2g38360	-0.87	-1.65	-0.91	-1.57	-0.84	-1.17	prenylated rab acceptor (PRA1) family protein
At3g48720	-1.23	-1.34	-0.93			-1.17	transferase family protein
At2g23620	-1.01	-1.68	-0.80			-1.16	esterase
At2g39450	-0.78	-1.17			-1.54	-1.16	cation efflux family protein
At5g46290	-1.35	-0.80	-0.85	-1.39	-1.43	-1.16	3-oxoacyl-[acyl-carrier-protein] synthase I
At4g28730	-1.77	-0.40	-1.32	-1.16		-1.16	glutaredoxin family protein
At2g30950	-0.49	-1.38	-1.04	-1.03	-1.85	-1.16	FtsH protease (VAR2)
At1g27480	-1.41	-1.63	-0.43			-1.16	lecithin:cholesterol acyltransferase
At5g08280	-0.60	-1.17	-0.56	-1.54	-1.92	-1.16	hydroxymethylbilane synthase
At4g16990	-0.17	-0.46	-1.25	-1.67	-2.23	-1.16	disease resistance protein
At3g54200	-0.95	0.11	-1.09	-1.65	-2.20	-1.15	expressed protein
At1g11820	-1.03	-0.18	-0.67	-1.26	-2.63	-1.15	glycosyl hydrolase family 17 protein
At5g67060	-1.44	-1.22		-0.31	-1.64	-1.15	basic helix-loop-helix (bHLH) family protein
At1g32610	-1.54	-1.25	-0.07	-0.74	-2.14	-1.15	hydroxyproline-rich glycoprotein
At3g57050	-0.44	-0.95	-0.80	-1.62	-1.92	-1.15	cystathionine beta-lyase
At5g04140	-1.44	-2.12	0.12			-1.15	glutamate synthase (GLU1)
At2g32870	-0.52	-0.42	-1.27	-2.62	-0.89	-1.14	meprin and TRAF homology domain-containing protein
At1g56050	-1.28	-1.37	-0.78			-1.14	GTP-binding protein-related
At5g22800	-0.72	-1.71	-0.54	-1.03	-1.71	-1.14	aminoacyl-tRNA synthetase family protein
At1g32200	-1.23	-0.32	-0.86	-1.49	-1.80	-1.14	glycerol-3-phosphate acyltransferase
At3g26540	-1.74	-1.85	-0.41		-0.57	-1.14	pentatricopeptide (PPR) repeat-containing protein
At1g79000	-0.81	-0.78	-0.58	-1.59	-1.94	-1.14	p300/CBP acetyltransferase-related
At5g44770	0.11	-0.76	-2.06		-1.85	-1.14	DC1 domain-containing protein
At3g25520	-0.67	-1.57	-0.36	-2.28	-0.80	-1.14	60S ribosomal protein
At2g37180	-0.64	-1.67	-2.09	-0.23	-1.05	-1.14	plasma membrane intrinsic protein 2C
At1g78140	-1.28	0.01	-1.20	-0.75	-2.41	-1.13	methyltransferase-related
At4g12150	-1.17	-1.40	-0.81			-1.13	zinc finger (C3HC4-type RING finger) family protein
At2g37170	-0.33	-1.51	-1.91	-0.08	-1.80	-1.13	plasma membrane intrinsic protein 2B
At2g13790	-1.09	0.15	-1.24	-1.81	-1.63	-1.12	leucine-rich repeat family protein
At2g44520	-1.36	-2.15	-1.40	-0.13	-0.58	-1.12	UbiA prenyltransferase family protein
At2g42320	-0.22	-1.10	-0.79	-1.38	-2.10	-1.12	nucleolar protein gar2-related
At5g64130	0.04	-1.10	-1.96	-1.62	-0.96	-1.12	expressed protein
At5g20720	-0.80	-1.08	-0.04	-2.13	-1.54	-1.12	20 kDa chaperonin
At1g29510	-2.41	-0.22	-1.38	-0.46		-1.12	auxin-responsive protein
At2g39795	-0.67	-2.19	-0.53	-1.57	-0.63	-1.12	mitochondrial glycoprotein family protein
At4g19200	0.19	-1.26	-0.85	-1.72	-1.92	-1.11	proline-rich family protein
At2g35840	-0.70	-2.31	-0.71	-0.72		-1.11	sucrose-phosphatase 1 (SPP1)
At1g47580	-1.22	-1.66	-0.45			-1.11	lipoyltransferase
At1g77940	-0.53	-1.69	-0.75	-1.90	-0.65	-1.11	60S ribosomal protein L30
At1g45230	-0.60	-1.71		-1.00		-1.10	defective chloroplasts and leaves protein-related
At5g13410	-0.24	-1.33		-1.74		-1.10	immunophilin
At5g01650	-0.59	-1.65	-0.35	-1.01	-1.90	-1.10	MIF family protein
At1g73300	-1.35	-1.05	-0.76	-1.04	-1.30	-1.10	serine carboxypeptidase S10 family protein
At2g27290	-0.51	-0.95	-0.42	-1.28	-2.33	-1.10	expressed protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At1g66430	-1.06	-0.69	-0.63	-2.02		-1.10	pfkB-type carbohydrate kinase family protein
At1g69790	-0.85	-0.59	-0.42	-2.54		-1.10	protein kinase
At3g21190	-1.13	-1.12	-1.05			-1.10	expressed protein
At3g54250	-1.76	-1.73	0.20			-1.10	mevalonate diphosphate decarboxylase
At3g24770	-0.92	-1.90	-0.46			-1.09	CLE41
At3g08920	-1.66	-1.63	0.02			-1.09	rhodanese-like domain-containing protein
At4g21445	-1.48	-1.47	-0.32			-1.09	expressed protein
At1g64255	-0.75	-1.49	-1.03			-1.09	SWIM zinc finger family protein
At5g14210	-0.85	-1.17		-1.24		-1.09	leucine-rich repeat transmembrane protein kinase
At5g16130	-0.29	-1.25	-0.50	-2.23	-1.17	-1.09	40S ribosomal protein S7
At2g43910	-0.62	-1.93	-0.63	-1.05	-1.20	-1.09	thiol methyltransferase
At5g26950	-0.94	-1.17	-1.14			-1.08	MADS-box family protein
At1g41855	-1.92	-0.61		-0.71		-1.08	hypothetical protein
At2g18300	-1.76	-0.95	-0.36	-1.24		-1.08	basic helix-loop-helix (bHLH) family protein
At1g63900	-0.50	-1.52	-1.62	-0.76	-0.99	-1.08	zinc finger (C3HC4-type RING finger) family protein
At5g16570	-1.38	-2.19	-1.05	-0.32	-0.44	-1.08	glutamine synthetase
At1g11840	-0.19	-1.45	-0.29	-1.76	-1.69	-1.08	lactoylglutathione lyase
At3g09600	-0.76	-0.63	-0.63	-0.91	-2.44	-1.07	myb family transcription factor
At4g00050	-2.09	-0.97		-0.16		-1.07	basic helix-loop-helix (bHLH) family protein
At2g44160	-0.67	-1.87	-0.68	-1.12	-1.04	-1.07	methylenetetrahydrofolate reductase 2
At1g72660	0.11	-0.65	-1.54	-2.21		-1.07	developmentally regulated GTP-binding protein
At5g45490	-0.92	-1.10	-0.62	-1.79	-0.93	-1.07	disease resistance protein-related
At5g10020	-0.63	-1.04	-0.36	-2.25		-1.07	leucine-rich repeat transmembrane protein kinase
At5g66470	-0.67	-0.89	-0.56	-1.13	-2.09	-1.07	expressed protein
At4g10300	-0.68	-0.66	-0.36	-1.72	-1.91	-1.07	expressed protein
At5g39910	-0.93	-1.00	-0.43	-1.42	-1.55	-1.07	glycoside hydrolase family 28 protein
At1g49975	-0.92	-1.68	-1.37	-0.30		-1.07	expressed protein
At2g41940	-0.46	-1.53	-0.38	-1.89		-1.07	zinc finger (C2H2 type) family protein
At2g07739	-1.43	-1.20	-0.57			-1.07	expressed protein
At1g48040	-1.38	-1.20	-0.62			-1.06	protein phosphatase 2C-related
At4g18730	-0.60	-1.04	-0.91	-1.96	-0.80	-1.06	60S ribosomal protein L11
At1g01620	-1.05	-0.57	-1.48	-0.41	-1.81	-1.06	plasma membrane intrinsic protein 1C
At4g34290	-1.30	-0.36	-1.53			-1.06	SWIB complex BAF60b domain-containing protein
At5g02710	-0.66	-0.66	-0.91		-2.02	-1.06	expressed protein
At1g29880	-0.30	-0.85	-2.01	-0.85	-1.28	-1.06	glycyl-tRNA synthetase
At1g21350	-0.18	-0.60	-0.65	-2.09	-1.77	-1.06	expressed protein
At1g32550	-0.85	-1.20	-0.44	-1.2	-1.59	-1.06	ferredoxin family protein
At5g19760	-0.63	-0.88	-0.20	-1.78	-1.79	-1.06	dicarboxylate/tricarboxylate carrier
At5g26880		-0.51	-1.07	-1.51	-1.14	-1.06	tRNA/rRNA methyltransferase
At3g61340	-0.57	-0.63	-0.49	-1.91	-1.68	-1.06	F-box family protein
At2g46455	-1.28	-0.63	-1.25			-1.06	cytochrome oxidase biogenesis protein-related
At5g10470	-0.87	-0.89	0.05	-2.50		-1.05	kinesin motor protein-related TH65 protein
At2g22125	-0.58	-0.55	-0.89	-1.70	-1.55	-1.05	C2 domain-containing protein
At3g06145	-1.44	-0.60	-0.52	-0.75	-1.96	-1.05	expressed protein
At1g33250	-0.94	-0.29		-1.93		-1.05	fringe-related protein
At1g07500	-0.87	-0.62	-0.87	-1.43	-1.47	-1.05	expressed protein
At3g26760	-1.35	-1.33	-0.47			-1.05	short-chain dehydrogenase
At2g42690	-0.28	-1.85	-0.57	-1.48	-1.07	-1.05	lipase
At2g18230	-0.94	-1.98	-0.70	-0.22	-1.39	-1.05	inorganic pyrophosphatase
At5g01180	-1.04	-0.75	-1.35			-1.05	POT family protein
At3g04760	-0.84	-1.30	-0.62	-0.33	-2.14	-1.04	pentatricopeptide (PPR) repeat-containing protein
At5g12940	-0.41	-0.90	-0.23	-2.11	-1.58	-1.04	leucine-rich repeat family protein
At5g07090	-0.24	-1.48	-0.78	-1.76	-0.95	-1.04	40S ribosomal protein S4 (RPS4B)
At4g13670	0.03	-1.67	-0.73	-1.50	-1.34	-1.04	peptidoglycan-binding domain-containing protein
At4g18590	-0.47	-0.66	-0.66	-2.02	-1.39	-1.04	expressed protein
At1g51220	-0.66	-2.22	-0.88	-0.18	-1.27	-1.04	zinc finger (C2H2 type) protein (WIP5)
At1g19050	-0.97	-1.22	-1.08	-1.28	-0.66	-1.04	two-component responsive regulator
At4g18780	-1.19	-0.95	-0.97			-1.04	cellulose synthase
At5g17040	-0.48	-1.34	-1.73		-0.61	-1.04	UDP-glucosyl transferase family protein
At1g78450	-0.96	-1.00	-0.49	-1.70		-1.04	SOUL heme-binding family protein
At4g25370	-0.55	0.02	-1.41	-1.31	-1.94	-1.04	Clp amino terminal domain-containing protein
At5g57800	-1.00	-1.69	-0.42			-1.04	CER1 protein
At3g50240	-0.51	-0.91		-1.69		-1.04	kinesin motor protein-related
At1g03870	-0.84	-1.39	-0.47	-0.83	-1.64	-1.04	fasciclin-like arabinogalactan-protein
At2g30390	-0.36	-1.08		-1.67		-1.04	ferrochelatase II
At4g15510	-0.57	-0.58	-0.34	-1.59	-2.07	-1.03	photosystem II reaction center
At2g02500	-1.19	-1.77	-0.13			-1.03	expressed protein
At1g05030	-0.66	-0.75	-1.03	-1.39	-1.30	-1.03	hexose transporter
At1g20810	-0.58	-0.46	-0.47	-1.32	-2.30	-1.03	immunophilin
At4g02530	-0.84	-1.80	-0.51	-1.05	-0.93	-1.03	chloroplast thylakoid lumen protein
At2g36570	-0.69	-0.88	-1.16	-1.36		-1.02	leucine-rich repeat transmembrane protein kinase
At3g62190	-0.51	-0.15	-1.34	-1.29	-1.82	-1.02	DNAJ heat shock
At1g74880	-0.82	-1.18	-0.52	-1.51	-1.08	-1.02	expressed protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At4g15740	-0.93	-0.56	-0.33	-2.27		-1.02	C2 domain-containing protein
At2g31790	-0.26	-1.71	-1.09			-1.02	UDP-glucosyl transferase family protein
At4g37210	-0.98	-1.17	-1.05	-0.88		-1.02	tetratricopeptide repeat (TPR)-containing protein
At2g19330	-1.23	-1.38	-0.31	-1.17	-1.01	-1.02	leucine-rich repeat family protein
At5g13140	-0.99	-0.80	-0.14	-1.46	-1.71	-1.02	expressed protein
At5g04680	-0.35	-1.39	-0.31	-1.64	-1.41	-1.02	expressed protein
At2g21140	-0.73	-0.57	-0.50	-1.32	-1.98	-1.02	hydroxyproline-rich glycoprotein
At2g03710	-0.58	-1.09	-0.42	-1.98		-1.02	MADS-box protein (AGL3)
At4g23630	-0.33	-1.42	-2.03	-0.70	-0.60	-1.02	reticulon family protein (RTNLB1)
At4g33865	-0.71	-1.38	-0.78	-1.47	-0.74	-1.01	40S ribosomal protein S29
At1g06200	-0.45	-0.64	-0.30	-1.66	-2.03	-1.01	expressed protein
At1g18730	-0.25	-0.97	-1.26	-1.28	-1.29	-1.01	expressed protein
At4g39970	-1.68	0.15	-0.66	-1.56	-1.31	-1.01	haloacid dehalogenase-like
At4g22830	-1.12	-0.62	-0.17	-1.18	-1.95	-1.01	expressed protein
At1g34470	-1.18	-0.94	-1.08	-0.60	-1.24	-1.01	permease-related
At2g40610	-1.13	-1.29	-0.61			-1.01	expansin (EXP8)
At1g21760	-1.28	-0.62	-0.86	-1.01	-1.26	-1.00	F-box family protein
At5g39290	-1.20	-1.36	-0.45			-1.00	expansin (EXP26)
At1g67320	-0.79	-2.22	-0.01			-1.00	DNA primase, large subunit family
At2g23010	-0.99	-1.01	-0.98	-0.31	-1.70	-1.00	serine carboxypeptidase S10 family protein
At2g42710	-0.69	-0.96	-1.34			-1.00	ribosomal protein L1 family protein
At3g27840	-1.08	-0.80	-0.18	-1.94		-1.00	50S ribosomal protein L12-2
At4g23740	-1.55	-1.19	-0.25			-1.00	leucine-rich repeat transmembrane protein kinase
At4g00800	-0.26	-1.06	-0.50	-1.72	-1.44	-1.00	expressed protein
At4g39770	-0.96	-1.58	-0.45			-1.00	trehalose-6-phosphate phosphatase
At4g11100	-0.22	-1.76	-0.70	-1.30		-1.00	expressed protein

A.3

Arabidopsis genes significantly up-regulated at 24 hpi after treatment of *Arabidopsis* leaf tissue with *Botrytis cinerea*. The experiment was replicated five times (Rep 1-5) and only genes significantly induced more than 2 fold (log of 1) on average are shown.

Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At4g20200	5.60	6.67	8.89			7.05	terpene synthase
At5g06730	4.56	7.22	8.04			6.61	peroxidase
At3g04720	5.04	7.41	7.97	7.04	5.53	6.60	hevein-like protein (HEL)
At3g58850	4.30	6.48	7.67			6.15	expressed protein
At5g61160	4.49	2.76	4.61	6.30	9.33	5.50	transferase family protein
At5g45890	3.13	5.90		7.33		5.45	senescence-specific SAG12 protein
At2g43620	3.85	3.86	3.79	8.85	5.97	5.26	chitinase
At2g43510	4.90	6.50	3.91	6.48	4.40	5.24	trypsin inhibitor
At4g16260	4.66	4.05	5.47	5.24	5.74	5.03	Beta-1,3-glucanase 1
At2g29350	4.74	5.83	5.74	4.81	2.97	4.82	tropinone reductase
At5g22300	4.25	2.81	4.57	6.01	6.00	4.73	nitrilase 4 (NIT4)
At1g10585	4.81	2.57		5.22	6.29	4.72	expressed protein
At5g56870	4.70	5.50	3.47	5.01	3.84	4.50	beta-galactosidase
At2g30750	4.62	2.68	5.32	4.65	5.02	4.46	cytochrome P450 71A12
At2g29470	4.97	2.69	2.8	5.71	5.89	4.41	glutathione S-transferase
At5g39610	4.03	4.30	2.44	5.94	5.07	4.36	no apical meristem (NAM) family protein
At1g73260	4.15	5.21	2.01	7.00	3.17	4.31	trypsin and protease inhibitor
At2g15360	3.40	1.82	3.74		8.20	4.29	hypothetical protein
At1g54100	4.27	4.96	3.72	4.66	3.75	4.27	aldehyde dehydrogenase
At3g54640	3.22	3.38	5.68	4.65	4.25	4.23	tryptophan synthase, alpha subunit
At2g30770	4.99	3.04	3.83	4.48	4.67	4.20	cytochrome P450 71A13
At3g59930	4.47	2.56	3.54	6.71	3.69	4.19	expressed protein
At2g36800	4.37	6.83	7.15	2.08	0.44	4.17	UDP-glucosyl transferase
At3g49620	4.10	2.89	2.44	5.65	5.78	4.17	2-oxoacid-dependent oxidase
At5g27980	2.73	4.21	5.56			4.17	seed maturation family protein
At2g36690	4.38	4.58	5.08	3.94	2.73	4.14	oxidoreductase, 2OG-Fe(II) oxygenase
At1g26420	3.32	3.55	3.78	4.92	4.80	4.08	FAD-binding domain-containing protein
At1g26400	3.75	4.57	5.22	3.22	3.59	4.07	FAD-binding domain-containing protein
At1g26380	4.01	3.29	4.08	4.33	4.33	4.01	FAD-binding domain-containing protein
At5g20230	4.05	3.75	5.56	3.15	3.44	3.99	Plastocyanin-like domain-containing protein
At2g39030	3.63	3.54	3.89	4.36	4.44	3.97	GCN5-related N-acetyltransferase
At4g33150	4.67	4.64	1.05	4.74	4.68	3.96	lysine-ketoglutarate reductase
At4g35770	4.95	3.18	0.82	5.18	5.53	3.93	senescence-associated protein (SEN1)
At3g55970	3.99	3.04	1.36	5.55	5.72	3.93	oxidoreductase, 2OG-Fe(II) oxygenase
At1g28230	1.94	5.67	4.10			3.90	purine permease (PUP1)
At1g02930	3.75	3.88	5.61	3.52	2.70	3.89	glutathione S-transferase
At2g26560	3.30	2.12	4.47	4.45	4.98	3.87	patatin
At3g22600	3.82	4.89	3.25	3.77	3.55	3.86	protease inhibitor/seed storage/LTP
At1g02920	3.89	3.52	3.70	3.91	4.20	3.84	glutathione S-transferase
At1g15520	3.40	2.31	3.94	4.65	4.92	3.84	ABC transporter family protein
At1g05700	3.26	4.19	3.91	3.51	4.24	3.82	leucine-rich repeat protein kinase
At2g28210	4.22	1.97	4.78	3.28	4.74	3.80	carbonic anhydrase
At1g08630	3.08	1.51	4.08	5.67	4.62	3.79	L-allo-threonine aldolase-related
At3g25250	4.25	5.00	6.10	1.71	1.81	3.77	protein kinase family protein
At1g10070	4.42	3.06	2.28	5.20	3.72	3.73	branched-chain amino acid aminotransferase 2
At3g49120	3.28	3.75	5.30	3.57	2.73	3.73	peroxidase
At1g32350	3.12	2.73	3.45	4.75	4.45	3.70	alternative oxidase
At4g32140	2.99	4.23	5.56	1.95		3.68	expressed protein
At1g25220	3.51	2.58	4.32	3.95	3.88	3.65	anthranilate synthase beta subunit
At4g08780	4.68	5.00	3.20	3.26	2.09	3.65	peroxidase
At4g11650	4.73	4.02	0.32	6.81	2.08	3.59	osmotin-like protein (OSM34)
At1g60740	3.46	1.85	4.02	4.51	4.05	3.58	peroxiredoxin type 2
At4g15610	4.90	4.11	2.60	4.65	1.62	3.57	integral membrane family protein
At4g37520	3.60	2.28	2.52	5.24	4.21	3.57	peroxidase 50
At3g60120	4.33	1.88	1.60	4.52	5.51	3.57	glycosyl hydrolase
At1g79680	2.72	1.18	2.54	4.67	6.68	3.56	Wall-associated kinase
At1g62380	3.96	3.83	2.19	5.29	2.50	3.55	1-aminocyclopropane-1-carboxylate oxidase
At3g51660	3.67	3.48	3.49	3.41	3.69	3.55	MIF family protein
At1g68620	2.97	2.16	2.33	4.20	6.07	3.55	expressed protein
At5g44420	-0.85	6.95	4.50	4.91	2.08	3.52	plant defensin protein (PDF1.2a)
At2g35730	2.89	1.89	1.88	5.39	5.52	3.51	heavy-metal-associated
At1g75110	1.49	3.94	5.12			3.51	expressed protein
At3g26200	3.69	2.51	2.51	4.82	4.02	3.51	cytochrome P450 71B22
At1g67980	3.86	2.29	2.64	4.87	3.86	3.51	caffeoyl-CoA 3-O-methyltransferase
At5g07010	3.26	3.08	2.74	4.13	4.28	3.50	sulfotransferase family protein
At5g67080	3.16	2.44	1.49	5.68	4.67	3.49	protein kinase family protein
At1g61800	2.70	1.23	3.14	4.99	5.33	3.48	glucose-6-phosphate/phosphate translocator
At4g21390	2.24			3.86	4.32	3.47	S-locus lectin protein kinase
At2g37770	3.17	1.39		5.80		3.45	aldo/keto reductase family protein
At1g74080	1.60	1.43		4.85	5.89	3.44	myb family transcription factor (MYB122)
At1g24909	3.52	1.82	4.19	3.56	4.09	3.43	anthranilate synthase beta subunit
At1g52200	3.63	1.79	3.25	3.47	4.99	3.43	expressed protein
At3g50210	3.88	3.14	3.18	3.75	3.16	3.42	2-oxoacid-dependent oxidase

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At5g14780	3.65	3.69	2.96	3.32	3.44	3.41	formate dehydrogenase
At3g44540	1.59	2.84	5.71			3.38	acyl CoA reductase
At3g48850	1.86	1.74	4.30	4.75	4.18	3.36	mitochondrial phosphate transporter
At3g53600	3.29	3.34	3.44		3.39	3.36	Zinc finger (C2H2 type) protein
At1g22400	2.81	2.47	5.17	3.40	2.90	3.35	UDP-glucosyl transferase
At5g02450	3.79	5.90	6.75	0.13	0.11	3.33	60S ribosomal protein L36
At3g16530		-0.21	5.62	3.95	3.93	3.32	legume lectin family protein
At1g59660	2.03	4.43	5.57		1.23	3.32	nucleoporin family protein
At1g05010	3.01	1.76	5.07	3.07	3.49	3.28	1-aminocyclopropane-1-carboxylate oxidase
At4g30270	3.52	3.49	2.53	3.71	2.92	3.23	MERI-5 protein
At4g34230	2.64	1.87	4.17	3.62	3.80	3.22	cinnamyl-alcohol dehydrogenase
At4g12490	3.80	4.50	-0.69	4.41	4.05	3.21	protease inhibitor/seed storage/LTP
At4g27450	2.19	2.25		4.8	3.58	3.20	expressed protein
At5g64120	2.98	2.65		4.00	3.13	3.19	peroxidase
At3g50770	2.80	1.51	3.39	4.17	4.07	3.19	calmodulin-related protein
At5g01210	2.90	2.31	2.49	4.34	3.84	3.17	transferase family protein
At2g25450	3.86	3.75	3.45	3.71	1.04	3.16	2-oxoglutarate-dependent dioxygenase
At3g26820	3.13	4.05	3.43	1.89	3.32	3.16	esterase/lipase/thioesterase
At3g01970		1.36	1.86	4.37	5.04	3.16	WRKY family transcription factor
At1g26390	3.54	3.33	-1.04	4.29	5.65	3.15	FAD-binding domain-containing protein
At1g54575	2.95	2.88	1.14	4.32	4.37	3.13	expressed protein
At1g25155	3.23	1.90	3.11	3.68	3.69	3.12	anthranilate synthase beta subunit
At1g69930	2.23	0.92	2.23	4.66	5.54	3.11	glutathione S-transferase
At4g02520	2.98	2.01	4.37	3.09	3.11	3.11	glutathione S-transferase
At2g02930	3.15	2.48	4.11	2.79	2.96	3.10	glutathione S-transferase
At1g21310	2.52	4.09	2.12	3.70	3.02	3.09	proline-rich extensin-like family protein
At3g04570	1.98	3.18	4.11			3.09	DNA-binding protein-related
At3g59480	2.23	3.64	3.32			3.06	pfkB-type carbohydrate kinase
At4g39950	3.30	1.24	2.82	4.14	3.79	3.06	cytochrome P450 79B2
At4g08770	3.54	2.12	1.63	4.31	3.68	3.05	peroxidase
At1g53070	2.59	2.98	3.15	3.27	3.22	3.04	legume lectin family protein
At2g35980	3.31	0.83	3.00	3.79	4.25	3.04	harpin-induced family protein
At1g75830	-0.48	6.92	0.82	3.49	4.38	3.03	plant defensin-fusion protein
At4g06634	2.50	3.29	5.37		0.93	3.02	Zinc finger (C2H2 type)protein
At3g26830	2.80	0.89	2.61	3.80	4.94	3.01	cytochrome P450 71B15
At1g51890	3.01	1.68	0.79	5.36	4.18	3.00	Leucine-rich repeat protein kinase
At3g28930	2.33	1.60	4.51	3.08	3.42	2.99	avrRpt2-induced AIG2 protein
At4g13310	2.06	1.50	2.92	3.97	4.45	2.98	cytochrome P450 71A20
At5g49780	3.36	7.26		0.43	0.86	2.98	leucine-rich repeat transmembrane protein kinase
At5g64905	2.60	1.77	1.52	4.02	4.91	2.96	expressed protein
At2g45220	2.42	2.17	0.47	4.81	4.95	2.96	pectinesterase family protein
At1g02220	3.17	1.43	3.15	4.08		2.96	no apical meristem (NAM) family protein
At5g11670	2.43	1.98	3.90	2.66	3.73	2.94	malate oxidoreductase
At1g02470	3.15	3.04	1.53	3.97	3.01	2.94	expressed protein
At5g11520	3.20	2.96	3.64	3.69	1.15	2.93	aspartate aminotransferase
At1g25083	3.21	1.50	2.75	3.59	3.55	2.92	anthranilate synthase beta subunit
At1g05680	2.99	1.28	0.06	7.36		2.92	UDP-glucosyl transferase
At5g13600	2.97	4.55	6.38	0.33	0.38	2.92	phototropic-responsive NPH3
At2g36200	1.33	1.76	5.63			2.91	kinesin motor protein-related
At1g22770	1.11		1.54		6.05	2.90	gigantea protein (GI)
At2g24180	3.12	2.17	2.69	3.44	3.03	2.89	cytochrome P450 family protein
At5g39520	2.69	2.55	0.39	5.24	3.57	2.89	expressed protein
At4g12480	2.98	2.99	2.26	1.26	4.92	2.88	protease inhibitor/seed storage/LTP
At3g26840	3.07	2.86	1.84	3.45	3.16	2.87	esterase/lipase/thioesterase
At4g37530	2.77	1.46	2.77	3.64	3.67	2.86	peroxidase
At2g35910	2.51	2.66	3.41			2.86	Zinc finger (C3HC4-type RING finger) protein
At4g39670	2.73	1.48	1.95	3.08	4.98	2.84	expressed protein
At1g11610	2.10	3.64	1.08	3.30	4.08	2.84	cytochrome P450
At1g54570	2.91	3.57	1.48	5.08	1.16	2.84	esterase/lipase/thioesterase
At3g49110	3.02	1.18	2.26	3.95	3.78	2.84	peroxidase 33
At2g31230	2.91	1.15	1.21	4.61	4.20	2.81	ethylene-responsive factor
At1g52890	2.08	1.41	1.32	3.63	5.55	2.80	no apical meristem (NAM) family protein
At2g47190	3.32	3.47	2.57	2.24	2.34	2.79	myb family transcription factor (MYB2)
At3g49630	2.05	2.22	2.21	3.21	4.23	2.78	2-oxoacid-dependent oxidase
At2g31390	1.65	3.69	3.61	2.74	2.23	2.78	pfkB-type carbohydrate kinase
At3g46660	2.51	0.70	0.95	4.97	4.77	2.78	UDP-glucosyl transferase
At5g57890	2.96	3.02	4.00	2.19	1.73	2.78	anthranilate synthase beta subunit
At4g36040	2.89	3.48	0.87	3.85	2.80	2.78	DNAJ heat shock
At2g33710	2.31	1.14	2.30		5.36	2.78	AP2 domain-containing transcription factor
At1g45145	2.79	2.11	3.31	2.57	3.05	2.77	thioredoxin H-type 5
At1g61820	3.27	2.14	-0.76	3.36	5.79	2.76	glycosyl hydrolase (BGLU46)
At5g17330	1.97	2.71	2.46	3.89		2.76	glutamate decarboxylase 1
At3g42810		1.46	2.35	4.45		2.75	hypothetical protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At5g06640	2.29	2.67	3.07	2.99	2.66	2.74	proline-rich extensin-like family protein
At1g76930	2.44	2.29	2.19	3.54	3.20	2.73	proline-rich extensin-like family protein
At4g24000	2.66	4.13	1.50	2.60		2.72	cellulose synthase
At4g15530	3.17	2.55	2.03	3.22	2.61	2.71	pyruvate phosphate dikinase
At1g66690	2.72	1.69	2.73	2.99	3.42	2.71	S-adenosyl-L-methionine:carboxyl methyltransferase
At5g01600	2.87	2.96	2.37	3.88	1.41	2.70	ferritin 1
At5g07000	3.65	2.35	1.31	3.30	2.80	2.68	sulfotransferase family protein
At2g28490	1.31	2.69	2.83		3.89	2.68	cupin family protein
At5g08790	2.96	1.53	2.57	2.93	3.36	2.67	no apical meristem (NAM) family protein
At2g29480	3.00	2.26	1.93	3.08	2.91	2.64	glutathione S-transferase
At2g16720	2.78	1.47	2.87	3.43		2.64	myb family transcription factor
At3g45300	3.35	2.36		2.39	2.45	2.64	isovaleryl-CoA-dehydrogenase
At1g80920	2.67	3.57	-0.05	2.81	4.18	2.64	DNAJ heat shock
At4g31970		1.21	2.30	4.93	2.04	2.62	cytochrome P450 family protein
At1g61560	1.83	1.07	2.46	4.40	3.33	2.61	seven transmembrane MLO
At4g05020	2.45	1.94	3.29	2.63	2.59	2.58	NADH dehydrogenase-related
At2g36380	2.21		2.84		2.68	2.58	ABC transporter family protein
At1g17745	3.21	1.04	1.00	4.05	3.57	2.57	D-3-phosphoglycerate dehydrogenase
At1g65690	2.99	1.06	0.97	4.28	3.55	2.57	harpin-induced protein-related
At5g20250	2.93	2.7	1.73	2.74	2.74	2.57	raffinose synthase family protein
At1g26410	2.90	1.84	2.96			2.57	FAD-binding domain-containing protein
At2g41380	3.00	1.53	0.99	4.04	3.26	2.56	embryo-abundant protein-related
At1g24807	2.75	1.18	2.66	3.05	3.17	2.56	anthranilate synthase beta subunit
At4g01870	2.96	1.00	2.50	3.21	3.14	2.56	tolB protein-related
At1g33780	2.99	2.94	3.16	1.93	1.77	2.56	expressed protein
At5g61950	1.52	2.00	0.72	6.00		2.56	ubiquitin carboxyl-terminal hydrolase-related
At3g26160	2.64	3.92	3.54	1.50	1.17	2.56	cytochrome P450 family protein
At4g03000	2.63	5.14	5.02	0.57	-0.59	2.55	expressed protein
At1g30780	2.37	4.32	3.73	-0.23		2.55	F-box family protein
At4g33540	2.83	1.66	1.24	4.13	2.86	2.54	metallo-beta-lactamase family protein
At4g17500	2.52	2.12	3.25	2.40	2.42	2.54	ethylene-responsive element-binding protein 1
At2g36780	2.74	2.50	1.20	3.72		2.54	UDP-glucosyl transferase
At4g01897	3.35	4.69	2.55	1.71	0.37	2.53	expressed protein
At4g37980	2.51	2.52	1.91	3.16	2.55	2.53	mannitol dehydrogenase
At3g26190	2.76	1.25	1.51	3.77	3.34	2.52	cytochrome P450 71B21
At3g49460	1.91	2.55		3.03	2.59	2.52	60S acidic ribosomal protein-related
At3g10320	3.90	1.72	2.30	2.08	2.59	2.52	expressed protein
At3g15500	1.27	1.28		4.41	3.10	2.52	no apical meristem (NAM) family protein
At4g16563	1.72	1.70	4.09			2.50	aspartyl protease
At3g03470	3.09	2.92	0.18	3.37	2.93	2.50	cytochrome P450
At2g40116	1.80	3.39	2.29			2.49	phosphoinositide-specific phospholipase C
At2g37750	3.44	2.95	1.06			2.49	expressed protein
At5g05600	2.14	2.69	1.80	3.19	2.55	2.47	oxidoreductase, 2OG-Fe(II) oxygenase
At2g02870	2.65	2.27	2.90	2.75	1.74	2.46	kelch repeat-containing F-box family protein
At5g57510	3.16	2.18	0.26		4.21	2.45	hypothetical protein
At5g58350	1.97	1.66	1.75	4.13	2.75	2.45	protein kinase family protein
At4g20930	2.00	1.48		3.50	2.81	2.45	3-hydroxyisobutyrate dehydrogenase
At4g24340	2.08	2.37	2.64	2.47	2.62	2.44	phosphorylase family protein
At2g18010	2.73	2.09	3.28	2.23	1.83	2.43	auxin-responsive
At5g38200	2.47	1.48	0.31	3.23	4.65	2.43	expressed protein
At3g01420	2.67	2.01	0.98	4.78	1.68	2.42	pathogen-responsive alpha-dioxygenase
At1g30710	0.95	2.94	3.35			2.41	FAD-binding domain-containing protein
At5g18130	2.39	1.88	1.43	3.13	3.21	2.41	expressed protein
At3g29035	3.11	4.04	1.12	2.86	0.90	2.41	no apical meristem (NAM) family protein
At1g03905	1.54	2.77	2.90			2.40	ABC transporter family protein
At5g07440	3.88	2.06	1.12	2.95	1.97	2.40	glutamate dehydrogenase 2
At4g18430	2.28	0.65	3.08	2.62	3.34	2.39	Ras-related GTP-binding protein
At3g54590	2.02	2.30	2.63	2.81	2.16	2.38	proline-rich extensin-like family protein
At4g12470	2.44	1.16	1.35	3.32	3.64	2.38	protease inhibitor/seed storage/LTP
At3g28940	1.99	1.27	4.65	1.63	2.33	2.37	avirulence-responsive protein
At1g74590	2.99	0.81	2.23	5.33	0.50	2.37	glutathione S-transferase
At1g69920	2.52	1.15	3.16	2.65	2.36	2.37	glutathione S-transferase
At1g13520	2.32	0.84		3.59	2.71	2.37	expressed protein
At3g27410	1.54	2.81	2.75			2.36	expressed protein
At2g29170	2.87	3.21	3.26	1.93	0.52	2.36	short-chain dehydrogenase/reductase
At3g29720	2.17	3.43	1.47			2.36	transferase-related
At3g44870	2.42	1.08	3.11	1.45	3.68	2.35	S-adenosyl-L-methionine:carboxyl methyltransferase
At5g67400	1.55	3.00	1.39	3.27	2.52	2.35	peroxidase 73
At5g54810	1.79	1.42	1.14	4.04	3.29	2.34	tryptophan synthase, alpha subunit
At4g30530	3.07	0.94	2.59	2.68	2.35	2.32	defense-related protein
At1g10140	2.13	1.22	2.16	2.89	3.22	2.32	expressed protein
At3g15356	0.11	0.08	2.88	4.54	3.99	2.32	legume lectin family protein
At4g04610	2.54	1.27	2.77	2.78	2.24	2.32	5'-adenylylsulfate reductase

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At1g77910	1.36	2.34	3.26			2.32	hypothetical protein
At3g55470	1.81	0.67		3.02	3.76	2.31	C2 domain-containing protein
At1g30700	3.42	1.29	1.69	3.23	1.90	2.31	FAD-binding domain-containing protein
At5g05730	3.37	1.04	2.32	2.28	2.50	2.30	anthranilate synthase
At3g01240	1.21	2.48	1.64	3.86		2.30	expressed protein
At2g18680	2.13	1.96	2.98	2.25	2.10	2.28	expressed protein
At4g21920	1.88	0.76	2.28	3.25	3.19	2.27	expressed protein
At3g54580	1.95	2.58	1.88	2.77	2.16	2.27	proline-rich extensin-like family protein
At4g21840	2.45	1.96	2.95	1.70		2.26	methionine sulfoxide reductase
At5g39580	2.08	1.81	1.64	3.52		2.26	peroxidase
At1g43160	2.00	0.94	1.01	3.53	3.81	2.26	AP2 domain-containing protein RAP2.6
At5g54140	2.61	1.73	3.10	2.24	1.55	2.25	IAA-amino acid hydrolase
At4g27070	1.98	2.07	2.08	2.37	2.73	2.24	tryptophan synthase, beta subunit 2
At5g17860	2.41	0.79	1.32	3.42	3.26	2.24	cation exchanger
At4g14020	1.84	2.70	1.14	2.23	3.28	2.24	rapid alkalinization factor
At4g06746	2.72	0.90	1.46	3.08	3.04	2.24	AP2 domain-containing transcription factor
At2g43150	2.01	2.18	2.39	2.45	2.15	2.24	proline-rich extensin-like family protein
At4g23680	2.76	1.43	1.09	3.67		2.24	major latex protein-related
At2g33150	3.09	2.29	1.53	2.78	1.44	2.23	acetyl-CoA C-acyltransferase
At1g58180	2.26	2.60	1.46	2.56	2.25	2.23	carbonic anhydrase
At1g66700	2.64	0.21	0.95	3.92	3.42	2.23	S-adenosyl-L-methionine:carboxyl methyltransferase
At5g64570	2.59	2.33	1.32	2.79	2.09	2.22	beta-d-xylosidase
At3g12500		0.52	0.98	5.17		2.22	basic endochitinase
At5g27420	2.13	0.77	1.54	2.71	3.94	2.22	Zinc finger (C3HC4-type RING finger) protein
At4g12290	2.20	1.07	2.67	3.03	2.10	2.21	copper amine oxidase
At4g13090	2.33	2.52	1.76	2.46	1.97	2.21	Xyloglucan:xyloglucosyl transferase
At5g17990	2.80	1.77	2.47	2.38	1.61	2.21	anthranilate phosphoribosyltransferase
At5g06860	2.21	1.76	1.52	2.88	2.65	2.20	polygalacturonase inhibiting protein 1
At1g17020	3.41	2.28	-0.27	4.54	1.06	2.20	oxidoreductase, 2OG-Fe(II) oxygenase
At3g47410	1.01	2.43	2.28	3.09		2.20	hypothetical protein
At3g53280	1.48	1.55	3.58			2.20	cytochrome P450 71B5
At5g04250	1.39	2.47	3.82	1.81	1.51	2.20	OTU-like cysteine protease
At3g04220	2.20	1.88	3.95	1.17	1.80	2.20	disease resistance protein
At3g52970	1.52	1.47	2.49	2.90	2.57	2.19	cytochrome P450 family protein
At5g32410	2.02	2.93	1.26	2.55	2.17	2.19	hypothetical protein
At4g35830	2.34	1.46	2.56	2.51	2.04	2.18	aconitate hydratase
At3g28210	2.46	1.56	1.27	3.04	2.54	2.17	Zinc finger (AN1-like) protein
At3g54150	1.55	1.11	1.17	2.55	4.50	2.17	embryo-abundant protein-related
At1g14540	2.22	2.34	1.18	2.17	2.93	2.17	anionic peroxidase
At1g10700	2.38	1.09	1.72	2.90	2.71	2.16	ribose-phosphate pyrophosphokinase 3
At3g23550	1.98	0.67	2.05	2.89	3.21	2.16	MATE efflux family protein
At1g03220	2.57	1.93	1.36	3.09	1.79	2.15	Extracellular dermal glycoprotein
At3g17790	2.04	1.34	1.20	3.41	2.71	2.14	acid phosphatase type 5
At1g01680	0.63	3.14	2.65			2.14	U-box domain-containing protein
At4g21830	1.95	1.25	0.95	2.60	3.94	2.14	methionine sulfoxide reductase
At3g02875	2.29	1.04	1.25	3.97		2.14	IAA-amino acid hydrolase 1
At2g44070	1.27	1.61	3.08	2.22	2.50	2.14	eukaryotic translation initiation factor 2B
At5g39130	2.33	1.43	0.82	3.45	2.64	2.14	Germin-like protein
At1g51800	1.20	2.37	1.49	1.80	3.81	2.13	Leucine-rich repeat protein kinase
At4g12735	4.05	2.26	1.13	3.68	-0.46	2.13	expressed protein
At3g54950	2.39		1.88	2.12		2.13	patatin-related
At3g58750	2.74	1.54	1.47	3.08	1.81	2.13	citrate synthase
At3g02040	1.86	-0.25		3.25	3.64	2.13	Glycerophosphoryl diester phosphodiesterase
At1g73950	1.8	3.18	4.18	0.33	1.11	2.12	Zinc finger (C3HC4-type RING finger) protein
At5g43450	1.63	0.50	2.14	2.70	3.63	2.12	2-oxoglutarate-dependent dioxygenase
At3g26210	2.40	2.77	1.18	1.89	2.35	2.12	cytochrome P450 71B23
At2g43570	1.78	1.10	2.08	2.86	2.71	2.11	chitinase
At5g40690	1.99	2.06		2.80	1.58	2.10	expressed protein
At1g15380	2.62	1.34	0.83	3.62		2.10	lactoylglutathione lyase
At1g62300	2.27	1.94	1.87	2.32		2.10	WRKY family transcription factor
At2g47210	1.69	3.93	4.57	0.45	-0.14	2.10	myb family transcription factor
At4g27300	1.18	0.97		2.08	4.16	2.09	S-locus protein kinase
At4g37770	2.27	3.44	1.71	1.47	1.58	2.09	1-aminocyclopropane-1-carboxylate synthase
At4g08410	2.23	2.20	1.42	2.60	2.00	2.09	proline-rich extensin-like family protein
At5g33290	2.07	1.80	0.85	3.26	2.46	2.09	exostosin family protein
At5g57220	1.57		2.06		2.63	2.09	cytochrome P450
At4g15200	1.00	2.75	2.50			2.09	formin homology 2 domain-containing protein
At3g28550	2.11	1.96	1.98	2.52	1.84	2.08	proline-rich extensin-like family protein
At5g62020	2.02	1.44		2.78		2.08	heat shock factor protein
At3g44310	2.28	2.72	3.24	1.86	0.23	2.07	nitrilase 1 (NIT1)
At1g49170	1.57	2.58	3.95		0.09	2.05	expressed protein
At5g27760	2.67	1.53	1.03	2.31	2.69	2.05	hypoxia-responsive
At1g74750	0.96	2.07	3.10			2.04	pentatricopeptide repeat-containing protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At2g20620	2.29	1.73	1.30	2.66	2.17	2.03	expressed protein
At1g61120	1.82	1.65	1.06	1.34	4.28	2.03	terpene synthase
At1g68630	1.80	1.58	1.52	3.21		2.03	expressed protein
At2g44080	2.47	1.02	0.86	2.16	3.60	2.02	expressed protein
At1g15415	1.07	0.67	0.75	3.71	3.89	2.02	expressed protein
At1g19020	2.10	1.01	2.28	1.81	2.89	2.02	expressed protein
At4g25810	2.05	1.20		3.04	1.77	2.01	Xyloglucan:xyloglucosyl transferase
At5g49450	0.13	0.51	1.72	5.14	2.56	2.01	bZIP family transcription factor
At2g17500	2.54	1.36	0.72	3.13	2.31	2.01	auxin efflux carrier
At1g21970	1.66	2.29	1.87	2.22		2.01	CCAAT-box binding transcription factor
At3g48450	1.95	0.63	0.32	3.88	3.26	2.01	nitrate-responsive NOI protein
At1g73480	1.92	2.93	0.92	1.96	2.29	2.00	hydrolase, alpha/beta fold
At2g24850	1.75	0.98	3.18	0.76	3.34	2.00	aminotransferase
At2g01950	1.06	2.91	5.10	0.67	0.25	2.00	Leucine-rich repeat transmembrane protein kinase
At2g33570	2.42	2.86	1.91	2.21	0.59	2.00	expressed protein
At1g30135	2.40	0.93	1.24	2.46	2.94	1.99	expressed protein
At3g51860	2.80	3.42	1.89	2.57	-0.72	1.99	cation exchanger
At4g38540	1.48	0.01	1.08	3.66	3.73	1.99	monooxygenase
At1g10190	1.54	2.30	0.68	3.44		1.99	expressed protein
At1g33030	2.78	0.37	0.66		4.15	1.99	O-methyltransferase family 2 protein
At2g45210	2.06	1.64	1.30	3.52	1.43	1.99	auxin-responsive protein-related
At2g03505	1.93	0.82	1.72	3.49		1.99	glycosyl hydrolase 17
At2g15960	2.57	0.61	-0.38	2.45	4.69	1.99	expressed protein
At4g21980	2.42	2.64	1.73	2.08	1.06	1.99	autophagy 8a
At1g48070	1.01	1.84	2.33	1.10	3.58	1.97	expressed protein
At1g76470	2.91	1.71	0.10	0.81	4.32	1.97	cinnamoyl-CoA reductase family
At2g24980	1.73	2.07	1.96	2.53	1.55	1.97	proline-rich extensin-like family protein
At3g51430	1.47	1.63	0.22	2.74	3.76	1.96	strictosidine synthase
At3g08760	1.35	2.35	1.81	2.15	2.15	1.96	protein kinase family protein
At5g26340	2.48	1.52	0.53	2.35	2.91	1.96	hexose transporter
At5g65110	2.38	1.41	1.20	2.70	2.08	1.95	acyl-CoA oxidase
At3g22200	2.85	0.65	1.52	2.59	2.15	1.95	4-aminobutyrate aminotransferase
At1g65240	1.89	2.89	1.69	1.68	1.62	1.95	aspartyl protease
At2g38870	2.14	0.73	1.97	2.68	2.24	1.95	protease inhibitor
At2g37430	2.35	0.03		1.82	3.58	1.94	Zinc finger (C2H2 type) protein
At1g14330	2.39	1.67	1.25	1.93	2.49	1.94	kelch repeat-containing F-box family protein
At5g63900	1.96	1.10		2.69	2.02	1.94	PHD finger family protein
At4g31950	2.41	1.31	-0.12	3.57	2.52	1.94	cytochrome P450 family protein
At1g47780	1.41	2.39	1.34	2.60	1.93	1.93	acyl-protein thioesterase-related
At5g24410	2.15	2.44	1.20			1.93	galactosamine-6-phosphate isomerase-related
At3g22460	2.79	3.05	3.55	0.23	0.03	1.93	cysteine synthase
At2g47270	1.41	1.64	1.37	1.45	3.79	1.93	expressed protein
At5g13820	2.17	0.81	1.20	3.07	2.39	1.92	telomeric DNA-binding protein 1
At1g62820	1.43	1.99	4.50	0.66	1.03	1.92	calmodulin
At1g14070	2.04	1.35	1.44	2.15	2.63	1.92	Xyloglucan fucosyltransferase
At5g03730	1.55	0.92	1.90	1.83	3.41	1.92	serine/threonine protein kinase
At5g54500	2.62	1.35	0.45	2.96	2.23	1.92	quinone reductase
At3g46280	2.67	1.89	1.28		1.86	1.92	protein kinase-related
At2g14610	1.89	1.13	2.29	2.16	2.12	1.92	pathogenesis-related protein 1
At3g48890	1.75	0.25	1.93	2.95	2.73	1.92	cytochrome b5 domain-containing protein
At2g05710	1.99	2.05	2.18	2.07	1.29	1.92	aconitate hydratase
At1g62225	1.09	2.84	1.81			1.91	expressed protein
At2g02710	3.03	1.14	1.48	2.80	1.11	1.91	PAC motif-containing protein
At1g23730	2.02		1.00		2.71	1.91	carbonic anhydrase
At1g58340	2.25	0.90	1.42	2.79	2.17	1.91	MATE efflux protein-related
At5g29210	1.14	1.72	2.86			1.91	hypothetical protein
At3g63270	3.33	1.36	1.30		1.64	1.90	expressed protein
At4g01895	3.05	2.92	0.26	1.57	1.72	1.90	SAR regulator protein
At5g59420	2.00	1.43	2.04	2.30	1.72	1.90	oxysterol-binding family protein
At5g54300	1.45	2.73	3.42	1.08	0.79	1.89	expressed protein
At4g16760	2.39	1.25	1.41	2.31	2.08	1.89	acyl-CoA oxidase
At1g67810	2.07		1.71	2.69	2.93	1.88	Fe-S metabolism associated
At1g73610	2.99	1.48	1.16			1.88	GDSL-motif lipase
At5g64370	2.10	1.82	2.10	2.38	0.99	1.88	beta-ureidopropionase
At1g29460	2.46	2.27	0.89			1.87	auxin-responsive protein
At1g55020	3.23	1.77	-0.20	3.28	1.26	1.87	lipoxygenase (LOX1)
At5g06630	2.06	1.04	2.10	2.30	1.84	1.87	proline-rich extensin-like family protein
At4g36990	2.16	1.13	2.34	1.35	2.33	1.86	heat shock factor protein 4
At4g22240	2.45	1.77	2.33	2.17	0.58	1.86	plastid-lipid associated protein PAP
At5g06870	2.61	2.61	1.51	0.80	1.78	1.86	polygalacturonase inhibiting protein 2
At2g46750	1.04		0.53	4.02		1.86	FAD-binding domain-containing protein
At4g19700	2.22	1.94	0.72	2.57		1.86	expressed protein
At3g06420	2.21	1.76	0.84	3.00	1.48	1.86	autophagy 8h

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At3g55880	1.92	1.00	1.41	2.50	2.45	1.86	expressed protein
At5g17340	2.17	1.69	1.74	1.96	1.72	1.86	expressed protein
At3g03870	1.82	1.93	0.82	2.03	2.67	1.85	expressed protein
At3g01650	2.18	0.76	1.80		2.65	1.85	copine-related
At2g34810	1.80	2.25	0.98	3.12	1.08	1.85	FAD-binding domain-containing protein
At2g13270	1.79	2.53	2.08	0.96	1.87	1.84	hypothetical protein
At2g37760	2.24	1.96	0.93	2.80	1.28	1.84	aldo/keto reductase family protein
At4g23190	1.64	0.35	1.88	2.28	3.03	1.84	protein kinase family protein
At1g76960	1.21	1.53	3.64	1.13	1.66	1.84	expressed protein
At2g36770	2.51	1.16	2.08	1.77	1.65	1.83	UDP-glucosyl transferase
At4g36640	1.93	0.46	1.34	2.90	2.52	1.83	SEC14 cytosolic factor family protein
At4g22470	1.92	0.92	1.16	2.17	2.98	1.83	protease inhibitor/seed storage/LTP
At2g38860	1.80	0.46	1.97	2.55	2.36	1.83	proteasel (pfpI)-like protein
At5g13080	2.12	0.57	0.39	2.81	3.24	1.83	WRKY family transcription factor
At4g34200	1.74	0.66	1.68	2.29	2.75	1.82	D-3-phosphoglycerate dehydrogenase
At3g26180	1.94	1.58	2.58	2.07	0.92	1.82	cytochrome P450 71B20
At4g33565	1.20	1.06	0.21	1.84	4.75	1.81	Zinc finger (C3HC4-type RING finger) protein
At2g29360	2.15	2.18	1.89	2.88	-0.04	1.81	tropinone reductase
At5g06070	2.26	2.69	1.10	1.19		1.81	Zinc finger (C2H2 type) protein
At1g23050	2.06	1.61	1.86	1.70		1.81	hydroxyproline-rich glycoprotein
At5g44990	1.60	1.76	2.48	2.18	1.02	1.81	hypothetical protein
At3g43682	2.55	3.99	1.70	0.48	0.30	1.80	hypothetical protein
At3g44300	2.27	2.42	0.65	3.21	0.46	1.80	nitrilase 2 (NIT2)
At4g08380	1.88	2.07	1.53	2.39	1.13	1.80	proline-rich extensin-like family protein
At5g35525	2.03	2.27	2.69	0.70	1.31	1.80	expressed protein
At2g23450	1.97	1.67	1.27	2.55	1.52	1.80	protein kinase family protein
At2g16630	1.72	2.42	1.71	2.03	1.09	1.80	proline-rich family protein
At5g62480	1.82	0.33	1.59	3.52	1.73	1.80	Glutathione S-transferase
At2g44340	1.18	3.88	3.12	0.33	0.44	1.79	VQ motif-containing protein
At3g13420	3.45	3.17	-1.26			1.79	expressed protein
At3g51895	2.14	1.27	1.71	2.83	0.98	1.79	sulfate transporter
At3g15150	2.30	2.81	0.25			1.78	expressed protein
At1g48020	1.59	1.38	2.37			1.78	invertase/pectin methylesterase inhibitor
At3g09350	1.74	1.30	2.44	1.62	1.81	1.78	armadillo/beta-catenin repeat
At1g21475	2.08	1.80	1.43			1.77	hypothetical protein
At5g27380	2.23	1.44	0.94	1.96	2.27	1.77	Glutathione synthetase
At5g60380	0.89	1.71	2.69			1.76	hypothetical protein
At3g51730	2.61	2.37	0.81	2.12	0.91	1.76	saposin B domain-containing protein
At2g40095	1.26	1.23		2.30	2.25	1.76	expressed protein
At3g23150	1.79	1.00	0.18	2.52	3.33	1.76	ethylene receptor
At2g27310	1.98	0.39	2.48	1.40	2.55	1.76	F-box family protein
At4g34590	0.95	0.14	2.68	2.80	2.21	1.76	bZIP transcription factor
At4g14690	1.64	1.90	2.87	1.84	0.53	1.76	chlorophyll A-B binding
At1g27420	1.74	2.28	1.25	1.74	1.76	1.75	kelch repeat-containing F-box family protein
At1g18570	1.67	0.19	1.86	1.50	3.53	1.75	myb family transcription factor (MYB51)
At4g36220	2.03	0.63	2.50	1.92	1.67	1.75	cytochrome P450 84A1
At4g19420	2.17	2.39	0.70	2.77	0.71	1.75	pectinacetylesterase family protein
At2g43590	0.77	1.15	0.92	2.34	3.56	1.74	chitinase
At1g79450	2.03	2.10	2.25	1.58	0.77	1.74	ligand-effect modulator 3
At5g26080	2.25	1.38	0.83	2.52	1.73	1.74	proline-rich family protein
At1g36763	2.00	1.82	1.09	2.26	1.54	1.74	hypothetical protein
At5g54390	1.40	3.51	2.75	1.16	-0.11	1.74	inositol monophosphatase
At1g04404	1.18		2.76	1.88	1.13	1.74	Zinc finger (C2H2 type) protein
At4g33420	1.60	1.41		2.77	1.17	1.74	peroxidase
At2g39050	1.58	1.38	1.29	2.05	2.37	1.73	hydroxyproline-rich glycoprotein
At1g19670	2.12	1.90	0.41	2.52	1.71	1.73	coronatine-responsive protein
At3g01290	1.36	0.79	3.35	1.15	2.01	1.73	band 7 family protein
At5g16980	2.46	1.47	1.53	2.39	0.79	1.73	NADP-dependent oxidoreductase
At3g18250	2.06	1.23	0.83	2.38	2.15	1.73	expressed protein
At5g39330	1.46	3.83	2.15		1.21	1.73	expressed protein
At1g80300	2.66	1.72	1.54	3.19	-0.49	1.72	chloroplast ADP
At1g32460	2.24	1.38	1.68	1.78	1.54	1.72	expressed protein
At3g50480	1.54	2.18	2.92	0.89	1.07	1.72	broad-spectrum mildew resistance RPW8
At5g64260	1.81	0.53		1.83	2.72	1.72	phosphate-responsive protein
At3g48020	1.69	2.35	1.42	1.91	1.22	1.72	expressed protein
At4g23885	1.87	1.00	2.27	2.35	1.09	1.72	expressed protein
At4g35170	0.91	2.24	2.00			1.72	hypothetical protein
At2g31250	1.28	2.77	2.15		0.68	1.72	glutamyl-tRNA reductase
At1g23720	1.83	2.04	1.21	2.02	1.47	1.72	proline-rich extensin-like family protein
At4g19370	2.02	0.70	0.14	3.38	2.32	1.71	hypothetical protein
At4g25900	2.17	1.33	1.12	2.12	1.81	1.71	aldose 1-epimerase family protein
At3g29034	2.18	1.52	-0.09	2.09	2.82	1.71	expressed protein
At2g32150	2.37	2.44	1.19	1.73	0.80	1.71	haloacid dehalogenase-like hydrolase

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At1g09500	2.59	2.63	0.65	2.48	0.17	1.70	cinnamyl-alcohol dehydrogenase
At2g30720	1.77	0.27	0.58	2.30	3.60	1.70	thioesterase family protein
At5g66760	2.25	1.07	1.78	2.35	1.06	1.70	succinate dehydrogenase
At4g08370	1.93	1.33	1.40	2.10	1.73	1.70	proline-rich extensin-like family protein
At1g55920	1.91	1.24	1.35	1.84	2.15	1.70	serine O-acetyltransferase
At5g43860	1.69	1.79	1.58	1.70		1.69	chlorophyllase
At1g60730	2.20	0.96	0.81	3.05	1.41	1.68	aldo/keto reductase family protein
At3g53180	1.80	1.37	1.62	1.83	1.77	1.68	glutamine synthetase
At4g04620	1.41	1.89	1.15	2.35	1.57	1.67	autophagy 8b
At2g31650	0.83	1.66	2.53			1.67	trithorax 1
At1g51760	2.80	0.90	1.12	1.70	1.85	1.67	IAA-amino acid hydrolase 3
At2g16790	1.43	0.70	1.45	0.96	3.82	1.67	shikimate kinase family protein
At3g57520	2.44	0.92	1.65	1.45	1.90	1.67	alkaline alpha galactosidase
At4g31860	1.76	1.30	2.35	1.13	1.81	1.67	protein phosphatase 2C
At3g21500	1.34	0.62	0.43	3.58	2.37	1.67	1-deoxy-D-xylulose 5-phosphate synthase
At3g23570	2.26	1.76	1.39	2.32	0.61	1.67	dienelactone hydrolase
At4g27740	1.96	2.27		1.65	0.79	1.67	Yippee family protein
At2g40580	0.79	2.77	0.85	1.54	2.38	1.67	protein kinase family protein
At3g22860	1.94	2.38	2.74	0.70	0.55	1.66	eukaryotic translation initiation factor 3 subunit
At1g36640	2.45	2.20	0.36	1.78	1.51	1.66	expressed protein
At1g70990	1.50	1.67	1.14	2.53	1.45	1.66	proline-rich family protein
At1g78380	2.09	1.32	2.93	2.27	-0.37	1.65	Glutathione S-transferase
At4g02380	1.35	1.65	3.15	0.94	1.11	1.64	late embryogenesis abundant 3
At2g14620	2.92	0.06	0.85	3.49	0.89	1.64	Xyloglucan:xyloglucosyl transferase
At4g34120	1.50	1.04	2.88	1.30	1.49	1.64	CBS domain-containing protein
At3g59940	1.79	0.83		2.30		1.64	kelch repeat-containing F-box family protein
At5g60530	1.67	1.16	2.08			1.64	late embryogenesis abundant protein-related
At2g47560	1.48	1.67	0.86	2.13	2.05	1.64	Zinc finger (C3HC4-type RING finger) protein
At2g04580	1.74	2.51	0.65			1.63	hypothetical protein
At4g30390	1.85	0.33	1.33	2.42	2.24	1.63	expressed protein
At5g09680	1.80	1.17	1.34	2.27	1.58	1.63	cytochrome b5 domain-containing protein
At3g23850	1.03	1.54	2.32			1.63	hypothetical protein
At1g74020	2.53	0.71	-0.20	3.60	1.52	1.63	strictosidine synthase
At1g13990	2.47	0.74	0.84	2.97	1.11	1.62	expressed protein
At4g37340	2.09	0.24	0.92	2.03	2.82	1.62	cytochrome P450 family protein
At5g38710	1.55	2.13	0.58	2.33	1.51	1.62	proline oxidase
At1g02230	1.57	1.83	1.47			1.62	no apical meristem (NAM) family protein
At2g43890	2.42	2.19	0.25			1.62	polygalacturonase
At5g01380	1.66	1.40	0.82		2.59	1.62	expressed protein
At1g69480	2.77	2.16		2.29	-0.75	1.61	EXS family protein
At1g27020	1.48	0.33	1.88	1.88	2.50	1.61	expressed protein
At5g54940	2.04	1.51	2.06	1.25	1.21	1.61	eukaryotic translation initiation factor SUI1
At1g59535	0.49	0.88		2.91	2.16	1.61	hypothetical protein
At1g55265	1.57	2.02	1.53	2.15	0.79	1.61	expressed protein
At2g29150	2.06	1.40	1.07	2.93	0.58	1.61	tropinone reductase
At1g20620	1.61	1.13	0.22	1.80	3.28	1.61	catalase 3 (SEN2)
At5g67310	2.18	0.72	0.94	1.50	2.70	1.61	cytochrome P450 family protein
At3g49210	1.56	0.57	0.45	2.88	2.55	1.60	expressed protein
At3g48990	1.92	1.36	2.06	1.81	0.84	1.60	AMP-dependent synthetase and ligase
At4g16000	1.53	1.89	2.94	0.17	1.45	1.60	expressed protein
At1g44800	2.15		1.02	2.26	0.94	1.59	nodulin MtN21
At3g13790	1.46	1.26	2.14	1.63	1.48	1.59	beta-fructosidase (BFRUCT1)
At2g15880			1.12	2.04	1.62	1.59	leucine-rich repeat family protein
At1g13360	1.93	1.22	1.34	0.49	2.96	1.59	expressed protein
At3g13950	0.25	0.53	0.62	3.55	2.99	1.59	expressed protein
At1g07670	1.67	2.12	2.82	1.14	0.18	1.59	calcium-transporting ATPase 4
At2g03170	1.68	0.78	1.92	1.69	1.85	1.58	E3 ubiquitin ligase
At5g25820	1.56	0.63		1.95	2.20	1.58	exostosin family protein
At1g58470	1.53	2.07	1.19	2.08	1.05	1.58	RNA-binding protein (XF41)
At2g37130	1.91	0.70	0.43	2.21	2.65	1.58	peroxidase 21
At5g23700	0.75	1.65	0.85	2.82	1.80	1.58	hypothetical protein
At4g15870	1.10	3.34	2.74	0.67	0.02	1.57	terpene synthase
At2g46680	2.66	1.09	1.73	1.57	0.80	1.57	homeobox-leucine zipper protein 7
At4g11530	0.83	0.61	1.28		3.56	1.57	protein kinase family protein
At1g72680	2.40	0.81	1.04	2.73	0.86	1.57	cinnamyl-alcohol dehydrogenase
At1g51790	0.74	1.10	1.58		2.86	1.57	Leucine-rich repeat protein kinase
At3g44720	1.99	0.66	1.66	1.51	2.00	1.57	prephenate dehydratase family protein
At3g47340	2.06	1.60	0.67	1.60	1.89	1.57	asparagine synthetase 1
At1g56130	1.15		0.67	2.60	1.83	1.56	leucine-rich repeat family protein
At5g18310	1.24	1.58	1.45	1.87	1.66	1.56	expressed protein
At1g73500	2.16	0.74	1.21	1.82	1.87	1.56	mitogen-activated protein kinase kinase
At3g51000	2.20	1.99	0.01	1.81	1.80	1.56	epoxide hydrolase
At2g47650	1.42	0.77	1.28	1.73	2.57	1.55	NAD-dependent epimerase

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At2g14170	2.17	1.29	0.16	1.97	2.18	1.55	methylmalonate-semialdehyde dehydrogenase
At1g62440	1.50	1.78	1.37	1.55		1.55	leucine-rich repeat family protein
At2g26440		0.16	0.84		3.64	1.54	pectinesterase family protein
At5g25260	1.66	0.59	1.90		2.02	1.54	expressed protein
At2g42790	2.14	0.86	0.14	2.53	2.05	1.54	citrate synthase
At1g70210	1.98	1.27	2.10	0.92	1.44	1.54	cyclin delta-1
At1g53580	2.37	1.51	0.60	2.21	1.01	1.54	hydroxyacylglutathione hydrolase
At1g61360	1.39	1.07	0.66	2.01	2.57	1.54	S-locus lectin protein kinase
At2g42890	2.12	1.38	-0.01	2.37	1.82	1.54	RNA recognition motif (RRM)-containing protein
At4g37150	2.62	0.81	1.18			1.54	esterase
At4g31380	0.88	1.85	1.48	1.60	1.86	1.53	hypothetical protein
At2g25625	1.88	1.73	0.68	2.42	0.96	1.53	expressed protein
At3g57410	1.78	1.61	1.35	1.46	1.45	1.53	Villin 3 (VLN3)
At3g50460	0.82	0.47	1.05	3.42	1.90	1.53	hypersensitive response protein 2
At2g02370	1.91	0.81	0.50	2.90		1.53	expressed protein
At5g38940	0.98	0.30		3.30		1.53	Germin-like protein
At1g47128	2.40	2.27	0.34	2.46	0.16	1.52	cysteine proteinase
At5g10520	1.47	0.64	0.49	3.50		1.52	protein kinase family protein
At3g28730	0.84	-0.07	0.66	3.05	3.13	1.52	structure-specific recognition protein 1
At5g53820	1.14	0.61	0.81	2.95	2.08	1.52	expressed protein
At3g51770	0.73	1.23	3.26	1.54	0.82	1.52	tetratricopeptide repeat
At4g08400	1.79	1.13	1.27	2.04	1.34	1.51	proline-rich extensin-like family protein
At5g09300	0.97	3.18	-0.04	1.94		1.51	2-oxoisovalerate dehydrogenase
At3g06690	1.90	1.87	1.58	1.81	0.40	1.51	acyl-CoA oxidase family
At4g18490	0.29	1.17	1.45		3.14	1.51	hypothetical protein
At4g29040	1.90	1.29	1.17	2.11	1.07	1.51	26S proteasome AAA-ATPase subunit
At1g62660	1.61	1.57	1.19	2.30	0.86	1.51	beta-fructosidase (BFRUCT3)
At1g09245	1.10	1.82	1.57	1.44	1.59	1.50	expressed protein
At5g49080	0.26	0.29	2.57	2.76	1.64	1.50	proline-rich extensin-like family protein
At5g15260	0.80			1.89	1.82	1.50	expressed protein
At1g68945	1.48	0.96	1.90	1.67		1.50	expressed protein
At3g21420	1.03	1.75	1.73			1.50	oxidoreductase, 2OG-Fe(II) oxygenase
At1g71030	1.68	1.26	1.06	1.65	1.84	1.50	myb family transcription factor
At3g24170	1.75	1.42	2.55	1.70	0.06	1.50	glutathione reductase
At3g01435	1.66	1.75	1.07			1.49	expressed protein
At1g22410	2.20	0.74	1.12	2.08	1.35	1.49	2-dehydro-3-deoxyphosphoheptonate aldolase
At1g56700	1.93	1.35	1.04	1.59	1.54	1.49	pyrrolidone-carboxylate peptidase
At3g19260	1.59	0.41	1.28	1.38	2.77	1.49	longevity-assurance (LAG1) family protein
At1g23550	1.11	1.57	3.71	0.62	0.41	1.48	expressed protein
At2g30860	2.06	-0.31	2.30	1.68	1.66	1.48	Glutathione S-transferase
At4g26970	1.79	1.99	1.65	0.53	1.41	1.48	aconitate hydratase
At3g45620	1.15		1.45	1.73	1.57	1.47	transducin family protein
At3g04320	1.88	0.86	0.71	2.43		1.47	trypsin and protease inhibitor
At1g25275	2.35	0.59	1.76	1.58	1.07	1.47	expressed protein
At4g21470	1.30	1.56	1.38	2.23	0.87	1.47	riboflavin kinase
At4g08470	1.15	0.66	1.15	1.74	2.64	1.47	mitogen-activated protein kinase
At1g41650	1.41	1.3	0.91	2.24		1.46	hypothetical protein
At2g44790	2.09	0.64	0.64	2.39	1.56	1.46	uclacyanin II
At5g39110	1.86	0.87	0.05		3.07	1.46	germin-like protein
At5g03220	1.78	1.29	0.96	1.96	1.32	1.46	transcriptional co-activator-related
At3g47540	1.69	0.81	0.53	2.11	2.17	1.46	chitinase
At5g18150	1.81	0.29	1.15	1.81	2.23	1.46	expressed protein
At3g60140	2.70	0.55	1.21	1.82	1.01	1.46	glycosyl hydrolase
At2g31570	2.21	1.69	0.82	2.07	0.50	1.46	glutathione peroxidase
At3g25760	1.54	0.83	1.96	0.88	2.08	1.46	early-responsive to dehydration stress protein
At1g73630	0.67	1.07	2.01	2.08		1.46	calcium-binding protein
At3g23270	1.46	2.08	0.83			1.45	regulator of chromosome condensation
At3g44550			1.41	0.83	2.13	1.45	acyl CoA reductase
At4g05633	1.16	1.16	1.66	1.63	1.65	1.45	hypothetical protein
At1g62570	2.13	0.25	0.36	3.06		1.45	flavin-containing monooxygenase
At4g25030	1.11	0.75	1.47	1.75	2.15	1.45	expressed protein
At2g33120			2.14	1.00	1.19	1.44	synaptobrevin-related protein
At1g26240	1.93	1.11	1.45	1.88	0.85	1.44	proline-rich extensin-like family protein
At1g17290	1.59	1.07	1.74	1.14	1.67	1.44	alanine aminotransferase
At5g20960	2.04	0.62	1.26	2.42	0.87	1.44	aldehyde oxidase 1
At3g06850	1.98	1.49	0.82	1.47		1.44	branched chain alpha-keto acid dehydrogenase E2
At4g17070	0.89	0.35	2.18	1.56	2.23	1.44	expressed protein
At2g41880	1.45	0.97	2.10	0.10	2.59	1.44	guanylate kinase 1 (GK-1)
At4g34138	2.18	1.06	1.07			1.44	UDP-glucosyl transferase
At5g28237	1.69	1.75	-0.11	2.43	1.44	1.44	tryptophan synthase, beta subunit
At3g27640	1.57	1.66	2.75		-0.24	1.44	transducin family protein
At4g37010	2.09	1.03	0.33	2.27	1.45	1.43	caltractin
At2g42990	1.67	1.09	1.30	1.88	1.23	1.43	GDSL-motif lipase

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At2g11490	1.51	1.44	0.88	1.91		1.43	hypothetical protein
At3g32902	1.67	1.49	0.74	1.82		1.43	hypothetical protein
At4g21810	1.05	1.32	3.44	0.37	0.96	1.43	Der1-like family protein
At3g20250	1.10	1.31	0.69	2.43	1.61	1.43	pumilio/Puf RNA-binding domain-containing protein
At1g61810	2.34	0.71	0.46	1.48	2.14	1.43	glycosyl hydrolase
At5g07990	1.68	2.78	0.43		0.81	1.43	flavonoid 3'-monooxygenase
At5g19550	1.84	1.61	1.35	1.58	0.75	1.42	aspartate aminotransferase
At5g67600	1.80	0.93	1.95	2.28	0.15	1.42	expressed protein
At1g28550	0.92	1.84	1.50			1.42	Ras-related GTP-binding protein
At3g21590	1.10	1.03	1.72	1.05	2.22	1.42	senescence/dehydration-associated
At5g45900	1.90	0.98	0.58	2.82	0.83	1.42	autophagy 7
At4g20970	1.00	0.26	0.91	2.78	2.17	1.42	basic helix-loop-helix (bHLH)
At5g22860	1.24	0.94	0.34	2.73	1.86	1.42	serine carboxypeptidase S28 family protein
At3g08640	1.61	0.12	0.54	2.12	2.70	1.42	alphavirus core
At5g02730	1.36	1.80	1.09			1.42	allergen V5/Tpx-1-related
At5g19440	1.54	0.71	0.42	2.52	1.88	1.41	cinnamyl-alcohol dehydrogenase
At2g29500	0.84	0.60	1.63		2.59	1.41	17.6 kDa class I small heat shock protein
At5g13490	1.49	-0.29	1.38	2.04	2.44	1.41	ADP, ATP carrier protein 2
At2g25590	1.47	2.24	1.27	1.96	0.12	1.41	agenet domain-containing protein
At5g02580	1.73	1.71	0.81	1.66	1.14	1.41	expressed protein
At1g70160	1.23	0.99	2.23	1.43	1.17	1.41	expressed protein
At3g51130	1.49		1.17	1.89	1.08	1.41	expressed protein
At2g18950	2.21	0.48	1.33	2.00	1.00	1.40	homogentisate phytylprenyltransferase
At1g02400	1.60	0.23	0.75	1.60	2.82	1.40	gibberellin 2-oxidase
At5g54067	0.86	1.2	2.15			1.40	expressed protein
At1g09970	1.48	0.27	1.30	1.49	2.47	1.40	Leucine-rich repeat transmembrane protein kinase
At5g57655	1.85	1.46	0.56	1.74	1.40	1.40	Xylose isomerase family protein
At1g35830	1.66	2.57	1.04	1.55	0.17	1.40	VQ motif-containing protein
At5g64250	1.69	0.59		2.31	1.00	1.40	2-nitropropane dioxygenase family protein
At3g59590	1.32	1.23	0.25		2.78	1.40	jacalin lectin family protein
At2g47600	2.12	1.20	0.20	2.41	1.05	1.40	magnesium/proton exchanger
At2g36220	1.99		0.50	1.76	2.72	1.39	expressed protein
At5g36880	1.81	0.36	0.67	2.27	1.84	1.39	acetyl-CoA synthetase
At3g48580	1.84	1.00	0.61	2.83	0.66	1.39	Xyloglucan:xyloglucosyl transferase
At3g25770	1.47	0.59	1.83	0.51	2.54	1.39	allene oxide cyclase
At3g62760	0.52	0.67	1.62	2.15	1.98	1.39	Glutathione S-transferase
At1g21000	2.02	1.23	0.99	1.71	0.99	1.39	Zinc-binding family protein
At5g24430	1.33	1.05	2.25	1.25	1.06	1.39	calcium-dependent protein kinase
At1g07400	0.97	1.34	0.65	1.44	2.52	1.38	17.8 kDa class I heat shock protein
At2g18350	1.28	1.40	0.46	2.40		1.38	Zinc finger homeobox protein
At1g53950	0.45	0.78	3.29	0.98	1.40	1.38	ubiquitin family protein
At2g40770	0.80	0.35	1.96	1.69	2.10	1.38	SNF2 domain-containing protein
At1g59950	1.08	1.13	0.37	2.56	1.75	1.38	aldo/keto reductase
At4g23180	1.68	0.90	1.22	1.93	1.15	1.38	receptor-like protein kinase 4
At1g04960	1.70	0.07	0.62		3.12	1.38	expressed protein
At3g09390	1.65	-0.24	0.49	2.35	2.63	1.37	metallothionein protein
At4g14250	1.57	1.71	1.60	1.62	0.36	1.37	UBX domain-containing protein
At2g30840	2.15	2.15	1.11		0.06	1.37	2-oxoglutarate-dependent dioxygenase
At5g63850	0.82	0.38		2.31	1.94	1.36	amino acid transporter 4
At3g07090	1.94	0.47	0.36	2.41	1.64	1.36	expressed protein
At2g19800	0.19	0.62	1.72	2.09	2.19	1.36	expressed protein
At1g11910	1.66	1.70	0.83	1.82	0.81	1.36	aspartyl protease
At3g59130	0.92	0.79	1.29	1.92	1.90	1.36	DC1 domain-containing protein
At2g29440	1.62	0.56	1.97	1.48	1.18	1.36	Glutathione S-transferase
At4g18950	1.85	0.52	0.78	1.47	2.17	1.36	ankyrin protein kinase
At1g21400	2.75	1.01	0.31		1.36	1.36	2-oxoisovalerate dehydrogenase
At5g04030		1.47	1.40		1.21	1.36	hypothetical protein
At1g26540	0.34	1.08	0.96	1.87	2.53	1.36	agenet domain-containing protein
At3g04300	1.40		1.87		0.79	1.36	expressed protein
At5g59570	0.94	1.23	1.90			1.36	myb family transcription factor
At5g61820	2.06	1.43	0.80	2.38	0.11	1.35	expressed protein
At5g19810	1.70	0.54	0.60	2.31	1.62	1.35	proline-rich extensin-like family protein
At4g21000	1.95	1.49	1.11	0.87		1.35	carbonic anhydrase
At2g25540	0.76	0.78	1.49	1.72	2.02	1.35	cellulose synthase
At4g24920	1.79	0.30	2.01	1.14	1.53	1.35	protein transport protein
At5g27600	1.78	0.70	0.14	2.49	1.65	1.35	AMP-binding protein
At2g46650	1.77	-0.59	1.77	1.71	2.10	1.35	cytochrome b5
At5g39050	1.34	0.20	-0.02	2.84	2.39	1.35	transferase family protein
At4g28085	1.26	0.96		1.41	1.77	1.35	expressed protein
At4g30490	1.95	0.77	0.86	2.14	1.00	1.35	AFG1-like ATPase family protein
At2g21500	1.85	1.35	1.67	0.76	1.10	1.34	expressed protein
At5g38560	0.89	2.66	1.83	0.56	0.79	1.34	protein kinase family protein
At5g65750	1.37	1.39	2.10		0.52	1.34	2-oxoglutarate dehydrogenase E1 component

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At3g21790	1.60	1.49	0.93			1.34	UDP-glucosyl transferase
At4g08098	0.78	0.43	2.18	1.77	1.53	1.34	hypothetical protein
At2g02650	1.09	1.19	1.73			1.34	reverse transcriptase-related
At5g24280	2.38	1.67	1.74	0.80	0.09	1.34	expressed protein
At5g05500	1.22	1.07	0.85	1.82	1.72	1.34	pollen Ole e 1 allergen and extensin
At2g27940	1.85	0.50	1.66			1.34	Zinc finger (C3HC4-type RING finger) protein
At5g63960	0.98	0.76	2.26			1.33	DNA-directed DNA polymerase
At2g17720	1.86	0.59	1.41	1.31	1.51	1.33	oxidoreductase, 2OG-Fe(II) oxygenase
At1g50890	0.54	1.65	1.95	1.44	1.09	1.33	expressed protein
At5g02490	1.14	0.49	2.16	1.60	1.28	1.33	heat shock cognate 70 kDa protein 2
At2g41410	2.16	1.32	0.39	1.55	1.23	1.33	calmodulin
At2g15220	2.13	0.96	0.81	3.07	-0.33	1.33	secretory protein
At5g06230	1.51	0.84	0.29	2.60	1.40	1.33	expressed protein
At1g74360	0.75	0.88	-0.24	2.29	2.93	1.32	Leucine-rich repeat transmembrane protein kinase
At1g13340	2.36	1.75	0.47	1.05	0.97	1.32	expressed protein
At2g44930	1.26	1.34	1.35	1.33		1.32	expressed protein
At3g30775	2.56	1.73	0.29	0.47	1.55	1.32	proline oxidase
At4g20460	1.36	1.20	1.62	1.18	1.23	1.32	NAD-dependent epimerase
At1g23070	0.89	0.85	2.20			1.31	hypothetical protein
At1g30620	0.84	1.13	1.26	1.69	1.64	1.31	UDP-D-xylose 4-epimerase
At4g08200	1.74	1.45	0.74			1.31	hypothetical protein
At1g19180	2.41	0.66	-0.47	1.71	2.23	1.31	expressed protein
At4g04480	1.40	0.16	0.57	1.75	2.65	1.31	hypothetical protein
At5g13180	1.78	1.41	0.60	1.57	1.16	1.31	no apical meristem (NAM) family protein
At5g47730	0.47	1.96	0.83	0.98	2.29	1.31	SEC14 cytosolic factor family protein
At1g71240	1.14	0.85	1.92			1.31	expressed protein
At5g14920	1.24	0.28	0.61	1.71	2.69	1.31	gibberellin-regulated
At5g24160	2.18	1.16	0.58			1.30	squalene monooxygenase 1,2
At2g31945	1.99	1.55	0.81	1.74	0.43	1.30	expressed protein
At5g10550	1.81	1.21	0.40	2.06	1.05	1.30	DNA-binding bromodomain-containing protein
At1g02530	1.37	1.13	0.92	2.04	1.05	1.30	multidrug resistance P-glycoprotein
At2g13550	1.36	1.48	0.38	1.76	1.54	1.30	expressed protein
At5g61450	1.36	0.65		1.90		1.30	2-phosphoglycerate kinase-related
At3g56390	1.00	0.63		1.55	2.03	1.30	hypothetical protein
At5g47460	0.87	0.14	1.68		2.51	1.30	pentatricopeptide repeat-containing protein
At1g15210	0.13	0.60	0.92	3.18	1.67	1.30	ABC transporter family protein
At1g01490	1.74	0.83	1.08	1.29	1.55	1.30	heavy-metal-associated
At3g05725	1.25	-0.03	0.96	2.61	1.69	1.30	expressed protein
At4g16200	0.76	0.15	1.84	2.48	1.23	1.29	suppressor-of-white-apricot
At1g24430	0.59	1.28	0.51	2.12	1.95	1.29	transferase family protein
At2g18170	1.45	0.51	0.50	2.10	1.90	1.29	mitogen-activated protein kinase
At5g35900	1.61	1.27	0.98			1.29	LOB domain family protein
At5g55560	1.01	1.08	0.98	1.92	1.46	1.29	protein kinase family protein
At4g01570	0.73	0.54	-0.01	2.46	2.71	1.29	pentatricopeptide repeat-containing protein
At4g33090	2.24	1.09	1.08	1.39	0.61	1.28	aminopeptidase M
At2g02515	1.57	1.10	0.73	2.07	0.95	1.28	expressed protein
At5g66590	1.80	1.16	0.80	1.9	0.75	1.28	allergen V5/Tpx-1-related
At5g63490	1.51	1.40	0.84	1.43	1.22	1.28	CBS domain-containing protein
At5g23600	0.96	2.60			0.28	1.28	tRNA 2'phosphotransferase
At5g40370	1.98	0.50	1.23	1.55	1.14	1.28	glutaredoxin
At4g37060	0.97	1.16	1.17	1.52	1.57	1.28	patatin
At3g26170	1.76	1.49	1.57	1.13	0.45	1.28	cytochrome P450 71B19
At4g35180	1.22	1.12	0.61	1.86	1.58	1.28	Zinc finger (C3HC4-type RING finger) protein
At4g40080	1.54	1.07	1.59	0.71	1.48	1.28	epsin N-terminal homology
At5g59220	1.68	1.13	1.32	1.51	0.74	1.28	protein phosphatase 2C
At4g39610	1.85	1.34	0.04	1.88		1.28	expressed protein
At1g26600	1.27	1.37	0.96	1.50		1.27	CLE9
At5g55200	1.77			1.01	1.03	1.27	co-chaperone grpE protein
At1g21250	1.00	0.28	1.94	1.32	1.81	1.27	Wall-associated kinase 1
At3g25110	1.94	0.93	0.16	2.03	1.29	1.27	acyl-[acyl carrier protein] thioesterase
At1g60690	2.16	1.05	1.32	1.59	0.21	1.27	aldo/keto reductase family protein
At3g06490	1.37	0.86	0.70	1.62	1.79	1.26	myb family transcription factor (MYB108)
At4g28090	1.65	0.91		1.34	1.16	1.26	multi-copper oxidase type I family protein
At5g52530	2.21	2.43	0.22	0.59	0.85	1.26	dentin sialophosphoprotein-related
At1g76150	1.53	0.27	0.82	2.43	1.26	1.26	maoC-like dehydratase
At1g33770	0.59	2.37	2.25	1.22	-0.13	1.26	protein kinase family protein
At1g33910	1.74	1.40	0.64			1.26	avirulence-responsive
At1g68450	1.91	1.07	0.80			1.26	VQ motif-containing protein
At5g13220	2.23	1.43	0.74	1.07	0.81	1.26	expressed protein
At1g69880	1.84	1.08	-0.19	2.12	1.43	1.26	thioredoxin
At3g06920	0.54	0.45	0.89		3.13	1.25	pentatricopeptide repeat-containing protein
At1g23800	1.45	1.50	0.41	2.43	0.49	1.25	aldehyde dehydrogenase
At5g40170	1.41	0.42	0.61	2.46	1.37	1.25	disease resistance

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At1g70300	1.88		1.27	0.60		1.25	potassium transporter
At5g35690	1.94	0.73	0.73	2.08	0.77	1.25	expressed protein
At3g22890	1.92	-0.03	1.69	1.60	1.08	1.25	sulfate adenylyltransferase 1
At5g58610	0.78	0.64	1.21		2.37	1.25	PHD finger transcription factor
At1g09560	1.71	0.72	0.81	1.27	1.74	1.25	germin-like protein
At5g26940	1.48	1.54	2.61	1.05	-0.44	1.25	exonuclease family protein
At4g27410	1.63	1.57	0.37	1.61	1.06	1.25	no apical meristem (NAM) family protein
At3g10985	1.71	0.36	0.12	1.58	2.46	1.25	Wound-responsive protein-related
At4g11840	1.36	0.59	1.25	1.34	1.69	1.24	phospholipase D gamma 3
At1g15670	1.24	1.06	-0.18	1.97	2.12	1.24	kelch repeat-containing F-box family protein
At1g40115	1.89	1.66	0.18			1.24	hypothetical protein
At4g30960	1.08	1.44	0.98	1.29	1.42	1.24	CBL-interacting protein kinase 6
At2g29250	1.65	-0.02	2.09			1.24	lectin protein kinase
At2g27830	1.6	1.20	0.52	1.27	1.61	1.24	expressed protein
At1g01650	1.76	0.94	0.69	1.19	1.61	1.24	protease-associated
At5g59360	1.86	1.64	-0.48	1.92		1.24	expressed protein
At3g44860	1.31	0.35	0.97	1.14	2.41	1.23	S-adenosyl-L-methionine:carboxyl methyltransferase
At3g04330	1.31	0.61	1.21	1.81		1.23	trypsin and protease inhibitor
At5g23510	1.04	-0.16	0.87	2.20	2.22	1.23	expressed protein
At2g39400	1.16	0.51		1.57	2.94	1.23	hydrolase, alpha/beta fold
At3g08690	1.35	0.54	0.76	1.73	1.77	1.23	ubiquitin-conjugating enzyme 11
At5g01520	0.76	1.75	0.01	2.40		1.23	Zinc finger (C3HC4-type RING finger) protein
At5g59540	1.32	0.92	0.54	1.35	2.02	1.23	oxidoreductase, 2OG-Fe(II) oxygenase
At1g27760	1.84	0.50	1.06	1.92	0.82	1.23	interferon-related developmental regulator
At1g33090	1.13	0.87	0.62	2.30		1.23	MATE efflux family protein
At3g23920	2.01	1.62	0.96	1.61	-0.08	1.23	beta-amylase
At4g10960	1.21	0.98	0.07	2.42	1.44	1.22	UDP-glucose 4-epimerase
At3g27470	1.81	2.32	1.22	1.32	-0.55	1.22	expressed protein
At5g41440	1.39	1.27	0.56	1.86	1.04	1.22	Zinc finger (C3HC4-type RING finger) protein
At5g16970	2.05	1.33	0.24	2.43	0.07	1.22	NADP-dependent oxidoreductase
At1g71880	1.55	1.20	1.29	1.44	0.62	1.22	sucrose transporter
At2g42140	1.79	1.38	0.23	1.79	0.92	1.22	VQ motif-containing protein
At5g46000	0.07	1.13	0.63	2.81	1.45	1.22	jacalin lectin family protein
At1g26680	0.76	1.31	1.59			1.22	transcriptional factor B3
At2g27200	1.64	-0.08	1.56	1.34	1.63	1.22	GTP-binding family protein
At3g22370	1.64	0.91	0.10	1.99	1.45	1.22	alternative oxidase 1a
At3g28810	0.9	0.49	1.53	2.93	0.23	1.22	hypothetical protein
At5g19700	1.45	0.70	0.92	1.95	1.06	1.22	MATE efflux protein-related
At1g26250	1.29	0.93	1.46	1.51	0.90	1.22	proline-rich extensin
At1g08070	0.65	0.49	1.04	1.92	1.98	1.22	pentatricopeptide repeat-containing protein
At2g40420	1.37	2.20	0.29	1.08	1.13	1.21	amino acid transporter
At1g62500	1.66	0.70	0.79	1.64	1.27	1.21	protease inhibitor/seed storage/LTP
At4g05320	1.86	0.04	1.25	2.11	0.80	1.21	polyubiquitin (UBQ10) (SEN3)
At5g08490	0.60	0.94	0.77	2.54		1.21	pentatricopeptide repeat-containing protein
At2g26530		0.01	0.73	1.39	2.71	1.21	expressed protein
At3g25780	1.33	0.79	1.24	0.62	2.08	1.21	allene oxide cyclase
At5g46875	1.03	0.81	1.79			1.21	hypothetical protein
At4g35210	0.76	0.35	1.69	2.20	1.04	1.21	hypothetical protein
At3g55910	1.51	0.88	1.22			1.21	expressed protein
At5g43430	1.39	1.76	0.73	1.28	0.88	1.21	electron transfer flavoprotein
At4g00070	0.82	0.95	0.26	2.67	1.33	1.20	Zinc finger protein-related
At1g27130	1.11	0.20	2.53	0.91	1.26	1.20	glutathione S-transferase
At5g26670	1.14	1.41	1.51	1.15	0.79	1.20	pectinacetylesterase
At4g04960	0.87	0.54	0.72	1.49	2.38	1.20	lectin protein kinase
At1g12910	1.25	2.26	1.81	1.33	-0.66	1.20	flower pigmentation protein
At1g77370	1.32	0.60	1.06	1.55	1.46	1.20	glutaredoxin
At5g58820	0.89	1.99	0.72			1.20	subtilase family protein
At1g48850	1.68	0.95	1.79	1.55	0.02	1.19	chorismate synthase
At2g40940	1.14	0.33	0.94	1.33	2.23	1.19	ethylene response sensor
At5g38650	1.76	1.39	0.89	1.48	0.45	1.19	proteasome maturation factor UMP1
At1g62760	1.96	1.46	1.06	1.30	0.18	1.19	invertase/pectin methylesterase inhibitor
At3g05950	1.00	0.50	0.86	1.69	1.91	1.19	germin-like protein
At1g28520	1.48	0.91	0.30	2.04	1.23	1.19	expressed protein
At1g03120			0.39	1.66	1.52	1.19	seed maturation family protein
At1g66950	1.13	0.43	1.42	1.42	1.56	1.19	ABC transporter family protein
At5g63130	1.41	-0.1	0.20	1.82	2.62	1.19	octicosapeptide/Phox/Bem1p
At1g13110	0.66	1.15	0.68	1.08	2.38	1.19	cytochrome P450 71B7
At5g01850	0.79	0.66	0.23	1.82	2.44	1.19	protein kinase
At1g69490	1.38	0.68	1.26	1.57	1.06	1.19	no apical meristem (NAM) family protein
At3g43684	1.66	0.52	0.49	2.05	1.23	1.19	hypothetical protein
At5g49280			0.14	1.47	1.95	1.18	hydroxyproline-rich glycoprotein
At1g73965	1.18	0.70	1.27	1.62	1.15	1.18	CLE13
At2g25735	0.93	-0.03	1.96	0.36	2.69	1.18	expressed protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At4g36410	1.05	2.00	0.75		0.94	1.18	ubiquitin-conjugating enzyme 17
At2g01650	1.15	0.62	1.41	1.54		1.18	Zinc finger (C2H2 type) protein
At5g57630	1.87	0.4	1.27			1.18	CBL-interacting protein kinase 21
At2g43410	1.49	1.47	1.38	2.07	-0.52	1.18	RNA recognition motif (RRM)-containing protein
At5g35560	0.97	0.39	2.90	0.89	0.75	1.18	DENN (AEX-3) domain-containing protein
At2g33110	1.23	0.25	2.29	0.96	1.16	1.18	synaptobrevin family protein
At4g39980	1.97	0.05	0.37	1.27	2.24	1.18	2-dehydro-3-deoxyphosphoheptonate aldolase
At5g28050	2.32	1.41	0.82	1.30	0.03	1.18	cytidine/deoxycytidylate deaminase
At2g20010	1.59	0.34	0.56	1.42	1.96	1.17	expressed protein
At1g29610	1.83	1.57	0.48	1.08	0.92	1.17	hypothetical protein
At4g24400	1.05		1.47	1.32	0.85	1.17	CBL-interacting protein kinase 8
At5g45110	1.13	0.54	0.72	1.55	1.92	1.17	ankyrin repeat family protein
At1g19310	0.99	0.2	0.45	1.18	3.03	1.17	Zinc finger (C3HC4-type RING finger) protein
At3g21630	1.26	-0.05	2.22		1.26	1.17	protein kinase family protein
At3g56330	0.70	2.33		0.87	0.79	1.17	N2,N2-dimethylguanosine tRNA methyltransferase
At1g12620	0.18	0.49	2.84			1.17	pentatricopeptide repeat-containing protein
At5g54840	0.84	0.74	0.38	2.33	1.56	1.17	GTP-binding family protein
At4g15260	1.22		1.60		0.69	1.17	UDP-glucosyl transferase
At5g14640	1.12	0.8	0.90	1.47	1.56	1.17	protein kinase family protein
At4g14310	0.62	0.79	0.06	1.48	2.88	1.17	peroxisomal membrane protein-related
At3g09810	1.07	2.07	1.96	0.51	0.22	1.17	isocitrate dehydrogenase
At2g39210	1.09	1.06	0.58	1.37	1.73	1.17	nodulin family protein
At1g45130	1.54	2.74	0.77	0.99	-0.21	1.17	beta-galactosidase
At3g11150	1.40	0.79	0.27	1.74	1.63	1.16	expressed protein
At4g26470	1.95	0.14		1.41		1.16	calcium-binding EF hand
At1g29290	1.78	0.41	0.74	1.49	1.40	1.16	expressed protein
At3g22060	1.65	1.14	-0.02	1.75	1.29	1.16	receptor protein kinase-related
At5g52770		0.50	0.40	1.64	2.11	1.16	heavy-metal-associated
At2g30240	1.03	0.76	1.68			1.16	cation/hydrogen exchanger
At1g59700	1.12	1.21	0.51	1.72	1.23	1.16	glutathione S-transferase
At1g75440	0.76	0.94	0.32	1.88	1.88	1.15	ubiquitin-conjugating enzyme 16
At3g10900	1.28	1.08	1.10			1.15	(1-4)-beta-mannan endohydrolase
At3g31940	0.82	1.24	1.41			1.15	hypothetical protein
At4g34550	0.22	0.71	1.37	1.45	2.00	1.15	expressed protein
At3g03540	1.61	0.69	1.25	1.14	1.07	1.15	phosphoesterase
At5g67390	0.13	1.14	2.34	0.90	1.24	1.15	expressed protein
At4g31330	1.25	1.12	1.08			1.15	expressed protein
At3g47480	1.17	1.04	2.29	0.60	0.64	1.15	calcium-binding EF hand
At3g19270	2.18	0.31	0.96			1.15	cytochrome P450 family protein
At1g49470	1.40	0.93	0.54	1.38	1.47	1.14	expressed protein
At1g48000	1.25	0.26	0.28	2.79		1.14	myb family transcription factor
At5g04180	0.83	1.22	1.67	1.20	0.80	1.14	carbonic anhydrase
At5g28110	0.91	1.19	1.11	1.50	1.01	1.14	hypothetical protein
At3g09010	1.42	0.42	0.26	1.55	2.05	1.14	protein kinase family protein
At1g10790	0.91	1.30	1.21			1.14	hypothetical protein
At1g80100	1.34	0.94	0.26	2.01		1.14	phosphotransfer family protein
At2g39220			1.37	1.01	1.04	1.14	patatin family protein
At4g19280	1.01	1.10	0.90	1.55		1.14	hypothetical protein
At2g32130	0.96	0.33	0.52	0.93	2.94	1.14	expressed protein
At4g24680	0.84		0.47	2.42	0.82	1.14	expressed protein
At4g13250	1.88	0.71	0.35	2.32	0.42	1.14	short-chain dehydrogenase
At5g67340	1.25	1.05	0.62	1.39	1.37	1.14	armadillo/beta-catenin repeat
At1g59870	1.25	0.27	1.47	0.81	1.87	1.13	ABC transporter family protein
At2g15760	1.38	0.33	1.10	1.17	1.68	1.13	calmodulin-binding protein
At5g22270	1.45	1.05	0.45	1.26	1.45	1.13	expressed protein
At1g56140	0.95	0.54	0.6	1.71	1.87	1.13	leucine-rich repeat family protein
At4g23170	1.50	0.77	0.68	1.33	1.39	1.13	protein kinase family protein
At1g65980	1.39	0.82	1.65	1.19	0.62	1.13	peroxiredoxin type 2
At3g18660	1.52	0.54	1.09	1.48	1.02	1.13	glycogenin glucosyltransferase
At2g31260	1.16	1.30	0.93			1.13	autophagy 9
At1g17190	1.48	0.48	1.07	0.93	1.69	1.13	glutathione S-transferase
At4g26930	1.14	1.94	1.42	0.46	0.68	1.13	myb family transcription factor (MYB97)
At5g13010	0.57	1.67	2.59	0.35	0.45	1.13	RNA helicase
At1g27300	1.76		0.25	1.69	0.80	1.13	expressed protein
At2g36950	1.69	0.33	0.02	2.21	1.38	1.13	heavy-metal-associated
At1g31240	1.37	1.42	0.26	1.83	0.74	1.12	expressed protein
At5g11950	1.60	1.46	0.64	1.28	0.64	1.12	expressed protein
At2g01490	1.89	0.86	0.82	1.38	0.66	1.12	phytanoyl-CoA dioxygenase
At2g22470	0.88	0.49		1.86	1.26	1.12	arabinogalactan-protein
At2g46140			0.75	1.20	1.42	1.12	late embryogenesis abundant protein
At1g48605	1.46	-0.18	0.76	1.58	1.99	1.12	halotolerance protein
At2g19160	1.53	1.05		0.78		1.12	expressed protein
At5g22460	0.99	0.40	0.40	2.27	1.54	1.12	esterase/lipase/thioesterase

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At3g25830	0.94	0.32	1.43	1.66	1.24	1.12	myrcene/ocimene synthase
At2g32240	1.88	-0.10	0.17	1.25	2.38	1.12	expressed protein
At2g30140	1.44	0.21	0.77	1.81	1.34	1.11	UDP-glucosyl transferase
At2g30360	2.00	0.25	0.72	1.16	1.43	1.11	CBL-interacting protein kinase 11
At1g10410	1.80	-0.09	0.60	1.88	1.38	1.11	expressed protein
At5g27920	0.79	0.66	0.75	2.08	1.28	1.11	F-box family protein
At2g28860	1.95	1.08	0.90	0.61	1.02	1.11	cytochrome P450 family protein
At4g21510	1.28	1.15			0.90	1.11	F-box family protein
At5g42890	1.18	0.61	1.29	1.52	0.96	1.11	sterol carrier protein 2
At1g73100	0.12	0.96	2.42	0.93		1.11	SET domain-containing protein
At5g46080	0.69	0.19	0.64		2.91	1.11	protein kinase family protein
At5g49900	1.44	0.91	1.45		0.63	1.11	expressed protein
At1g65820	1.66	0.87	0.40	1.73	0.88	1.11	microsomal glutathione s-transferase
At4g28088	2.08	1.17	0.07			1.11	hydrophobic protein
At5g04040	1.56	0.44	0.51	1.85	1.15	1.10	patatin-related
At1g22930	1.80	1.50	0.42	1.36	0.44	1.10	T-complex protein 11
At2g32200	1.41	0.80	1.34	1.05	0.91	1.10	hypothetical protein
At3g05200	0.91	0.87	1.92	0.49	1.31	1.10	Zinc finger (C3HC4-type RING finger) protein
At2g25280	0.96		0.57	1.77		1.10	expressed protein
At5g06030	0.48	1.01	1.20	1.49	1.33	1.10	self-incompatibility protein-related
At1g01340	0.61	1.70	1.18	1.36	0.64	1.10	cyclic nucleotide-regulated ion channel
At2g41730	1.48	0.77	1.19	1.59	0.46	1.10	expressed protein
At3g45540		0.04	0.38	2.40	1.57	1.10	Zinc finger (C3HC4-type RING finger) protein
At3g07700	1.22	1.30	1.11	1.45	0.40	1.10	ABC1 family protein
At1g50790	0.50	2.20	0.86	1.19	0.73	1.09	hypothetical protein
At5g42370	-0.56	1.59	1.57	1.53	1.34	1.09	expressed protein
At3g49790	1.58	0.27			1.42	1.09	expressed protein
At1g51930	0.72	1.12	1.44			1.09	Zinc finger (C3HC4-type RING finger) protein
At1g32585	1.87	1.17	0.24			1.09	VQ motif-containing protein-related
At3g54620	1.35	0.66	0.31	1.71	1.43	1.09	bZIP transcription factor
At2g39660	1.43	0.14	0.70	1.42	1.78	1.09	protein kinase
At4g04760	2.07	0.83	0.72	0.88	0.95	1.09	sugar transporter family protein
At1g23120	1.90	0.70	1.19	1.15	0.50	1.09	major latex protein-related
At5g02430	0.50	1.26	0.39	2.19		1.09	WD-40 repeat family protein
At3g11402	1.43	0.87	1.74	0.61	0.77	1.08	DC1 domain-containing protein
At5g36930	0.35	1.64	2.05	0.90	0.48	1.08	disease resistance protein
At1g78670	1.89	0.31	-0.26	1.84	1.64	1.08	gamma-glutamyl hydrolase
At1g21245	0.85	0.52	0.79	1.09	2.16	1.08	Wall-associated kinase-related
At2g15490	1.76	0.82	1.02	2.01	-0.21	1.08	UDP-glucosyl transferase
At2g41690	0.93	0.95	1.37			1.08	heat shock transcription factor
At3g09440	1.03	0.57	-0.03	2.02	1.81	1.08	heat shock cognate 70 kDa protein 3
At3g24090	1.73	1.75	1.15	0.50	0.27	1.08	glucosamine fructose-6-phosphate aminotransferase
At2g23830	1.10	0.83	0.56	1.23	1.68	1.08	Vesicle-associated membrane protein
At5g56350	1.89	0.52	0.44	1.86	0.69	1.08	pyruvate kinase
At5g06510	1.55	0.42	1.26			1.08	CCAAT-binding transcription factor
At3g04350	0.28	1.66	1.86	0.77	0.83	1.08	expressed protein
At4g05640	1.38	1.03	0.82			1.08	hypothetical protein
At1g56250	0.65	0.79	1.78			1.07	SKP1 interacting partner 3-related
At3g53160	1.12	0.91	0.20	1.35	1.79	1.07	UDP-glucosyl transferase
At3g08720	1.49	0.49	0.35	1.33	1.71	1.07	serine/threonine protein kinase
At4g21380	0.67	0.21	0.63		2.78	1.07	S-locus protein kinase
At1g74400	-0.28	0.99	1.68	2.19	0.79	1.07	pentatricopeptide repeat-containing protein
At1g20110	1.09	0.78	1.52	0.53	1.45	1.07	Zinc finger (FYVE type) protein
At1g09240	1.68	2.44	0.13	1.07	0.05	1.07	nicotianamine synthase
At3g46210	0.94	1.23	1.59	0.80	0.78	1.07	3' exoribonuclease
At3g28720	1.31	1.42	0.81	1.33	0.48	1.07	expressed protein
At4g16146	1.70	-0.47	0.94	2.10		1.07	expressed protein
At1g60680	1.58	0.75	0.62	1.51	0.87	1.07	aldo/keto reductase family protein
At4g36480	1.16	0.26	0.85	1.43	1.63	1.07	aminotransferase class I and II
At5g59720	1.05	0.61	0.26	1.68	1.72	1.06	18.1 kDa class I heat shock protein
At3g24503	1.35	-0.39	2.01	1.00	1.35	1.06	aldehyde dehydrogenase
At5g45410	1.48	0.88	-0.01	1.64	1.32	1.06	expressed protein
At5g26770	0.97	0.86	0.45	1.83	1.20	1.06	expressed protein
At2g16500	1.05	0.53	0.25	1.64	1.84	1.06	arginine decarboxylase 1
At3g57280	1.51	0.08	0.65	1.41	1.67	1.06	expressed protein
At3g22570	0.34	2.22		1.68		1.06	protease inhibitor/seed storage/LTP
At1g50390	1.62	0.73	0.73		1.14	1.06	fructokinase-related
At3g58470	0.59	1.96	1.38	0.84	0.52	1.06	expressed protein
At4g26740	0.58	1.03	2.00	0.99	0.68	1.06	embryo-specific protein 1
At1g32450	1.84	1.68	-0.41	1.77	0.40	1.06	POT family protein
At2g45300	1.37	1.00	0.93	1.12	0.86	1.05	3-phosphoshikimate 1-carboxyvinyltransferase
At2g27380	1.18	0.24	0.81	1.54	1.49	1.05	proline-rich family protein
At1g56010	0.99	0.83	0.35	1.62	1.48	1.05	transcription activator NAC1

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At2g29490	1.43	0.52	0.68	1.65	0.99	1.05	Glutathione S-transferase
At2g24410	0.45	1.13	1.38	1.14	1.16	1.05	hypothetical protein
At1g33660	1.66	0.62	1.29	1.34	0.33	1.05	peroxidase family protein
At5g19680	0.91	1.28	2.30	0.84	-0.08	1.05	leucine-rich repeat family protein
At1g03290	1.24	0.72	0.91	1.34	1.04	1.05	expressed protein
At5g08450	0.81	0.52	0.83	1.33	1.75	1.05	expressed protein
At1g03740	0.91	0.10	0.55	1.44	2.25	1.05	protein kinase family protein
At1g31820	1.67	0.55		1.54	0.43	1.05	amino acid permease
At5g63740	0.71	0.82	1.86	0.81		1.05	zinc finger protein-related
At1g14370	1.78	0.26	0.20	1.71	1.29	1.05	protein kinase (APK2a)
At2g32850	1.34	0.97	0.64	1.34	0.96	1.05	protein kinase family protein
At5g51250	1.87	1.26	0.14	0.72	1.26	1.05	kelch repeat-containing F-box family protein
At4g00240	1.18	0.43	1.16	1.21	1.25	1.05	phospholipase D beta 2
At1g03380	1.48	1.21	1.07	1.08	0.39	1.05	expressed protein
At1g53890	1.05	0.91	0.06	1.88	1.32	1.05	expressed protein
At4g20350	1.34	0.80	1.33	0.96	0.79	1.04	expressed protein
At3g55410	1.35	0.81		1.04	0.97	1.04	2-oxoglutarate dehydrogenase E1 component
At3g28060	0.82	1.22	1.57	1.15	0.45	1.04	nodulin MtN21
At1g51780	0.51	1.25	0.40	1.04	2.01	1.04	IAA-amino acid hydrolase 5
At2g21160	1.71	1.00	1.27	1.07	0.15	1.04	translocon-associated protein
At5g56030	1.39	0.07	1.48	1.23	1.03	1.04	heat shock protein 81-2
At1g75390	0.39	0.38	0.32	1.72	2.39	1.04	bZIP transcription factor
At5g15600	1.34	0.75	0.77	1.19	1.13	1.04	expressed protein
At2g02700	1.71	1.69	0.41		0.33	1.04	DC1 domain-containing protein
At5g37000	0.38	0.93	1.81			1.04	exostosin family protein
At5g60300	0.96	0.60	0.15	1.73	1.74	1.04	lectin protein kinase family protein
At2g05030	0.75	0.51	0.47	1.44	2.00	1.03	hypothetical protein
At2g27150	0.60	1.39	0.41	1.74		1.03	aldehyde oxidase 3
At5g44575	1.06	0.46	0.75	1.08	1.82	1.03	expressed protein
At5g57400	1.65	1.16	0.28	1.28	0.80	1.03	hypothetical protein
At2g32905	0.20	1.26	1.47		1.19	1.03	hypothetical protein
At5g59070	0.66	1.35	0.16	1.45	1.55	1.03	glycosyl transferase
At3g02270	0.57	0.89	1.02	1.73	0.96	1.03	eIF4-gamma/eIF5/eIF2-epsilon
At5g26330	1.24	1.13	1.26	1.04	0.48	1.03	plastocyanin-like domain-containing protein
At5g57887	1.19	1.24	0.62	0.68	1.42	1.03	expressed protein
At5g52080	2.57	0.70	-0.01	0.91	0.96	1.03	expressed protein
At1g71490	0.48	2.02	1.91	0.34	0.39	1.03	pentatricopeptide repeat-containing protein
At4g21790	1.66	0.28	1.24	1.47	0.50	1.03	transmembrane protein-related
At4g20190	1.06	0.94	1.08			1.03	hypothetical protein
At5g23410	1.01	0.81	1.26			1.03	expressed protein
At4g19880	1.46	0.65	0.89	1.12	1.00	1.02	Glutathione S-transferase-related
At1g08620	0.38	0.9	1.79	0.84	1.22	1.02	transcription factor jumonji
At1g13210			0.40	1.08	1.60	1.02	haloacid dehalogenase-like
At2g37940	1.27	-0.07	0.52	1.02	2.39	1.02	expressed protein
At5g55130	1.35	0.92	1.24	1.12	0.50	1.02	molybdenum cofactor synthesis protein 3
At3g42520	1.64	1.51	0.53	0.62	0.81	1.02	hypothetical protein
At3g55550	0.89	1.38	2.36	0.33	0.15	1.02	lectin protein kinase
At1g69450	1.75	1.11		0.20		1.02	early-responsive to dehydration protein-related
At5g07830	0.91	1.28	0.47	1.43		1.02	glycosyl hydrolase 79
At5g54870	1.72	1.22	0.08	1.61	0.48	1.02	expressed protein
At3g54940	0.56	1.66	0.92	1.21	0.75	1.02	cysteine proteinase
At1g07473	1.27	1.50	0.51	0.79		1.02	hypothetical protein
At1g72870	0.27	1.25	0.88	1.68		1.02	disease resistance protein
At1g15125	0.56	0.34	1.36	1.33	1.51	1.02	S-adenosyl-L-methionine:carboxyl methyltransferase
At5g38600	0.97	0.73	-0.31	2.69		1.02	proline-rich spliceosome-associated
At3g26280	0.86	1.28	0.80	1.72	0.42	1.02	cytochrome P450 family protein
At2g11520	0.84	0.60	1.46	0.99	1.19	1.02	protein kinase family protein
At3g15200	1.28	0.95	0.82			1.02	pentatricopeptide repeat-containing protein
At5g09440	1.13	0.59	1.38	1.13	0.85	1.02	phosphate-responsive protein
At5g50140	1.79	0.94	0.32			1.01	ankyrin repeat family protein
At1g60380	0.76	1.22	1.07			1.01	apical meristem formation protein-related
At4g33050	0.87	-0.17	0.87	1.02	2.48	1.01	calmodulin-binding
At5g40720	0.57	0.54		1.46	1.50	1.01	expressed protein
At3g52710	0.67	2.20	1.62	0.42	0.16	1.01	expressed protein
At3g01200	1.46	0.25	1.43	0.92		1.01	expressed protein
At1g11480	0.92	1.30	0.41	1.62	0.82	1.01	eukaryotic translation initiation factor-related
At5g38530	1.13	0.37	1.22	2.05	0.30	1.01	Tryptophan synthase-related
At2g25270	0.88	0.36	1.47	1.33		1.01	expressed protein
At1g61370	0.37	0.70	0.14	1.80	2.05	1.01	S-locus lectin protein kinase
At1g18380	0.62	0.45	1.37		1.61	1.01	expressed protein
At4g30920	1.66	-0.10	2.23	0.66	0.60	1.01	cytosol aminopeptidase
At1g80865	1.14	1.91	0.54	0.66	0.79	1.01	expressed protein
At4g25720	0.74	1.56		0.42	1.32	1.01	glutamine cyclotransferase

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At1g04780	1.10	0.66	0.76	1.51		1.01	ankyrin repeat family protein
At4g07390	1.68	0.83	0.78	1.11	0.64	1.01	PQ-loop repeat family protein
At4g19530	0.71	0.91	0.91	0.99	1.51	1.01	disease resistance protein
At5g61030	0.40	1.13	1.55	1.12	0.82	1.00	RNA-binding protein
At2g29370	1.29	0.42	1.21	1.25	0.86	1.00	tropinone reductase
At5g16410	0.36	0.52	1.67	1.48	0.99	1.00	transferase family protein
At4g18300	1.28	0.06	0.92		1.75	1.00	eIF4-gamma/eIF5/eIF2-epsilon
At4g18690	0.68	0.84	1.48			1.00	hypothetical protein
At2g25200	1.31	0.98	0.81	1.30	0.61	1.00	expressed protein
At1g69280	0.86	1.32	0.82			1.00	expressed protein
At3g52510	1.29	0.71	0.77	1.30	0.91	1.00	F-box family protein
At3g53210	0.41	1.15	1.24	1.21	0.98	1.00	nodulin MtN21
At4g10895	0.35	0.55	0.51	1.84	1.72	1.00	expressed protein
At4g37320	1.17	0.82	-0.22	2.22		1.00	cytochrome P450 family protein
At5g04930	0.98	0.81	0.46	1.18	1.56	1.00	phospholipid-transporting ATPase 1
At1g05340	1.37	0.61	0.96	1.30	0.74	1.00	expressed protein

A.4 *Arabidopsis* genes significantly down-regulated at 24 hpi after treatment of *Arabidopsis* leaf tissue with *Botrytis cinerea*. The experiment was replicated five times (Rep 1-5) and only genes significantly repressed more than 2 fold (log of 1) on average are shown.

Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At3g60370			-9.06	-5.36	-2.77	-5.73	immunophilin
At1g65230	-2.95	-2.91	-11.06		-3.60	-5.13	expressed protein
At5g38430	-3.24	-5.17	-7.23	-5.39	-4.10	-5.03	RuBisCO small subunit 1A
At5g38420	-3.03	-4.39	-6.66	-5.79	-4.97	-4.97	RuBisCO small subunit 2B
At4g37930	-4.17	-5.03	-5.21	-5.61	-4.69	-4.94	glycine hydroxymethyltransferase
At1g65490	-3.11	-3.65	-5.94	-5.65	-5.56	-4.78	expressed protein
At1g67090	-3.15	-4.13	-6.48	-5.48	-4.13	-4.67	RuBisCO small subunit 1A
At3g25805	-1.86	-2.17	-10.77		-3.55	-4.59	expressed protein
At1g12900	-3.78	-3.85	-5.10	-4.98	-4.66	-4.47	GAPA-2
At5g38410	-2.96	-4.00	-6.22	-4.86	-4.22	-4.45	RuBisCO small subunit 3B
At1g72610	-3.78	-4.68	-4.98	-4.91	-3.46	-4.36	germin-like protein
At5g14750		0.59	-6.76		-6.86	-4.35	myb family transcription factor
At5g35630	-2.89	-4.61	-5.01	-4.69	-4.15	-4.27	glutamine synthetase
At4g39330	-3.52	-5.19	-3.85	-4.48		-4.26	mannitol dehydrogenase
At4g22570	-3.45	-2.93	-4.60	-5.05	-5.07	-4.22	adenine phosphoribosyltransferase
At1g75900	-1.62	-1.70	-9.21			-4.18	Extracellular lipase 3
At4g29630	-0.85	-0.78	-8.05	-2.81	-8.33	-4.16	cytidine deaminase
At3g26060	-2.79	-3.8	-3.21	-6.37	-4.28	-4.09	peroxiredoxin Q
At5g13930	-3.35	-2.63	-1.35	-6.33	-6.64	-4.06	chalcone synthase
At5g65470		-0.88	-8.82	-2.46		-4.05	expressed protein
At5g45680	-2.60	-4.74	-3.85	-4.88	-4.05	-4.03	FK506-binding protein 1
At5g36700	-3.13	-4.05	-2.35	-5.19	-4.84	-3.91	phosphoglycolate phosphatase
At2g42220	-2.78	-4.51	-4.60	-4.65	-3.03	-3.91	rhodanese-like
At1g20340	-2.87	-3.75	-6.01	-4.07	-2.80	-3.90	plastocyanin
At2g39730	-2.75	-3.52	-5.09	-3.64	-4.47	-3.89	RuBisCO activase
At5g41050	-2.42	-3.63	-3.42		-5.88	-3.84	expressed protein
At5g58260	-2.98	-2.83	-2.49	-5.22	-5.33	-3.77	expressed protein
At2g21210	-2.91	-1.17	-1.18	-6.24	-7.32	-3.76	auxin-responsive protein
At3g62030	-3.04	-4.39	-2.12	-4.98	-4.02	-3.71	peptidyl-prolyl cis-trans isomerase
At1g14150	-2.28	-4.06	-1.67	-5.71	-4.80	-3.70	Oxygen evolving enhancer 3
At3g14415			-4.09	-3.36	-3.58	-3.68	(S)-2-hydroxy-acid oxidase
At1g74670	-3.04	-3.00	-3.98	-6.29	-2.07	-3.68	gibberellin-responsive protein
At1g60390	-1.19	-1.07	-8.69			-3.65	BURP domain-containing protein
At3g56290	-1.88	-2.47	-4.21	-2.65	-7.02	-3.65	expressed protein
At5g05580	-2.09			-4.74	-4.06	-3.63	omega-3 fatty acid desaturase
At5g16920		0.39	-4.92		-6.29	-3.61	expressed protein
At2g21330	-2.36	-3.05	-2.64	-4.83	-5.11	-3.60	fructose-bisphosphate aldolase
At4g37800	-2.46	-2.48	-8.91	-3.04	-1.09	-3.59	xyloglucan:xyloglucosyl transferase
At1g42970	-2.73	-2.66	-2.82	-4.61	-5.14	-3.59	GAPB
At5g64040	-2.07	-3.05	-6.91	-3.47	-2.46	-3.59	photosystem I reaction center
At5g08260	-1.79	-1.63	-8.73	-3.75	-2.01	-3.58	serine carboxypeptidase S10 family protein
At3g52720	-2.90	-3.29	-2.21		-5.90	-3.58	carbonic anhydrase
At1g35680	-2.53	-3.11	-4.27	-5.11	-2.84	-3.57	50S ribosomal protein L21
At1g20020	-2.98	-3.95	-2.91	-4.68	-3.31	-3.57	ferredoxin NADP(+) reductase
At5g39210	-2.24	-3.64			-4.77	-3.55	expressed protein
At5g15230	-1.65	-5.11	-4.41	-4.76	-1.69	-3.53	gibberellin-regulated protein 4
At1g32470	-3.19	-2.99	-2.32	-4.56	-4.56	-3.52	glycine cleavage system H protein
At5g23060	-3.80	-3.16	-2.17	-3.86	-4.54	-3.51	expressed protein
At2g05310	-2.34	-2.62	-5.17	-3.91	-3.46	-3.50	expressed protein
At4g26530	-1.98	-2.86	-3.13	-5.13	-4.37	-3.49	fructose-bisphosphate aldolase
At5g18020	-2.31	-1.13	-7.84	-2.68		-3.49	auxin-responsive protein
At3g54050	-3.09	-2.47	-2.47	-5.35	-4.00	-3.48	fructose-1,6-bisphosphatase
At5g15660		-1.35	-6.08		-3.00	-3.48	F-box family protein
At1g04420	-1.36	-2.23	-6.93	-4.16	-2.68	-3.47	aldo/keto reductase family protein
At5g38520	-2.40	-2.76	-2.72	-4.72	-4.62	-3.45	hydrolase, alpha/beta fold family protein
At4g13500	-2.49	-2.82	-3.82	-4.72	-3.37	-3.44	expressed protein
At1g18420	-2.44	-2.56	-5.32			-3.44	expressed protein
At5g36790	-3.03	-2.98	-1.36	-5.21	-4.54	-3.43	phosphoglycolate phosphatase
At1g51400	-1.89	-3.53	-6.66	-3.00	-2.00	-3.42	photosystem II 5 kD protein
At5g44220		0.01	-3.82	-5.45	-4.41	-3.42	F-box family protein
At5g24300	-1.58	-0.80	-9.69	-2.82	-2.07	-3.39	starch synthase
At4g34620	-1.95	-3.45	-4.23	-4.31	-3.01	-3.39	ribosomal protein S16 family protein
At4g29905	-2.36	-1.72	-3.04	-4.10	-5.61	-3.37	expressed protein
At3g22231	-2.61	-3.29	-3.39	-4.27	-3.25	-3.36	expressed protein
At3g51510	-2.40	-3.37	-3.12	-4.74	-3.12	-3.35	expressed protein
At1g22630	-2.83	-2.16	-3.36	-3.63	-4.72	-3.34	expressed protein
At4g33010	-2.82	-3.08	-2.90	-4.15	-3.76	-3.34	glycine dehydrogenase
At5g06090	-2.07	-2.65	-3.41		-5.17	-3.32	phospholipid/glycerol acyltransferase
At2g24270	-2.33	-2.37	-6.82	-3.39	-1.69	-3.32	NP-GAPDH
At2g06850	-2.73	-3.27	-4.33	-5.68	-0.44	-3.29	xyloglucan:xyloglucosyl transferase
At1g19150	-2.69	-2.55	-0.65	-5.28	-5.26	-3.28	chlorophyll A-B binding protein
At5g35480	-3.40	-3.80		-3.35	-2.57	-3.28	expressed protein
At2g16370	-0.64		-4.36	-6.21	-1.89	-3.27	bifunctional dihydrofolate reductase-thymidylate synthase

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At5g24314	-1.85	-1.88	-5.30	-4.06	-3.24	-3.27	expressed protein
At4g28750	-2.48	-3.10	-6.50	-3.59	-0.61	-3.26	photosystem I reaction center subunit IV
At1g10280	-0.08	-2.80	-6.88			-3.25	expressed protein
At3g51820	-2.73	-1.62	-3.85	-3.92	-4.13	-3.25	chlorophyll synthetase
At4g35130	-2.70	-3.56	-5.14	-2.86	-1.97	-3.25	pentatricopeptide (PPR) repeat-containing protein
At4g38860	-3.88	-1.57	-2.74	-5.50	-2.55	-3.25	auxin-responsive protein
At1g29430	-2.50	-1.37	-4.41	-4.53	-3.37	-3.24	auxin-responsive family protein
At4g22490	-1.38		-7.10	-3.54	-0.91	-3.23	protease inhibitor/seed storage/LTP
At4g25050	-2.02	-1.80	-4.31	-3.72	-4.32	-3.23	acyl carrier family protein
At1g48350	-2.15	-3.24	-3.06	-4.68	-3.00	-3.22	ribosomal protein L18 family protein
At3g13275	0.37	-0.17	-7.30	-5.65	-3.33	-3.21	expressed protein
At4g34650	-0.90	-1.12	-7.61			-3.21	farnesyl-diphosphate farnesyltransferase 2
At5g45950	-2.38	-2.42	-3.56	-3.28	-4.33	-3.19	GDSL-motif lipase/hydrolase family protein
At3g21190	-2.21	-2.10	-4.89		-3.53	-3.18	expressed protein
At2g01590	-1.94	-2.26	-0.04	-6.29	-5.24	-3.15	expressed protein
At1g78840	-2.53	-3.55	-2.30	-3.97	-3.36	-3.14	F-box family protein
At4g21280	-2.29	-3.02	-3.12	-4.36	-2.79	-3.12	oxygen-evolving enhancer protein 3
At5g63930	-2.31	-2.98	-3.23	-3.16	-3.80	-3.09	leucine-rich repeat transmembrane protein kinase
At3g21870	-1.53	-1.56			-6.18	-3.09	cyclin family protein
At1g75690	-2.41	-2.33	-2.54	-4.44	-3.70	-3.08	chaperone protein dnaJ-related
At4g28660	-2.48	-1.56	-1.02	-4.49	-5.82	-3.07	photosystem II reaction centre W
At5g14060	-2.15	-2.06	-2.99	-4.13	-4.04	-3.07	aspartate kinase
At5g33240		-0.59	-4.71	-3.89		-3.06	hypothetical protein
At2g46820	-2.29	-2.12	-3.45	-4.43	-2.99	-3.05	expressed protein
At3g02730	-2.20	-2.70	-3.41	-3.55	-3.40	-3.05	thioredoxin
At4g27700	-2.38	-2.23	-3.64	-3.57	-3.44	-3.05	rhodanese-like domain-containing protein
At1g30380	-2.34	-3.35	-3.62	-3.86	-2.05	-3.04	photosystem I reaction center subunit psaK
At1g32060	-2.73	-3.98	-1.61	-3.58	-3.29	-3.04	phosphoribulokinase (PRK)
At2g43030			-2.82	-3.71	-2.57	-3.03	ribosomal protein L3 family protein
At4g24770	-2.85	-2.57	-2.76	-3.76	-3.20	-3.03	31 kDa ribonucleoprotein
At1g64680	-2.89	-4.21	-1.24		-3.71	-3.01	expressed protein
At1g75680	-2.44	-1.91	-3.88	-5.42	-1.43	-3.01	glycosyl hydrolase family 9 protein
At1g44575	-2.11	-2.56	-1.03	-4.66	-4.69	-3.01	photosystem II 22kDa protein
At2g06520		-1.75	-4.56	-3.47	-2.25	-3.01	membrane protein
At5g02120	-1.95	-2.80	-1.88	-3.65	-4.74	-3.00	thylakoid membrane one helix protein
At1g71500	-1.79	-2.59	-4.80	-2.76	-3.06	-3.00	Rieske [2Fe-2S] domain-containing protein
At1g30360	-1.86	-4.12	-3.40	-4.34	-1.28	-3.00	early-responsive to dehydration stress protein
At4g04640	-1.70	-3.18	-3.70	-3.63	-2.77	-3.00	ATP synthase gamma chain 1
At4g05180	-2.67	-2.93	-6.03	-3.22	-0.03	-2.98	oxygen-evolving enhancer protein 3
At5g55710	-1.10	-1.30	-3.61	-2.83	-6.03	-2.97	expressed protein
At1g02320	-2.57	-2.21	-3.12	-3.11	-3.79	-2.96	hypothetical protein
At3g47070	-1.04	-2.17	-2.94	-4.35	-4.31	-2.96	expressed protein
At4g18370	-2.35	-2.28	-2.69	-4.35	-3.08	-2.95	protease HhoA
At2g26910	-2.62	-2.10	-3.05	-4.36	-2.61	-2.95	ABC transporter family protein
At4g18480	-1.94	-2.99	-3.02	-3.33	-3.46	-2.95	magnesium-chelatase
At5g27780	-3.19	-3.02	-2.62			-2.94	auxin-responsive family protein
At1g66100	-2.41	-1.67	-3.95	-4.22	-2.43	-2.94	thionin
At2g10940	-2.85	-2.17	-2.36	-4.57	-2.69	-2.93	protease inhibitor/seed storage/LTP
At3g06895	-2.72	-1.35	-4.72			-2.93	expressed protein
At3g50820	-2.15	-2.43	-2.49	-3.88	-3.62	-2.91	oxygen-evolving enhancer protein
At3g21960	-0.59	-2.09	-7.40	-2.24	-2.20	-2.91	receptor-like protein kinase-related
At2g21710	-2.46	-3.14	-3.51	-2.58	-2.81	-2.90	mitochondrial transcription termination factor-related
At4g34260	-1.25	-0.34	-5.01	-5.62	-2.27	-2.90	expressed protein
At4g05090	-1.39	-1.92	-5.84		-2.39	-2.89	inositol monophosphatase family protein
At1g70760	-2.22	-3.26	-1.47	-4.27	-3.18	-2.88	inorganic carbon transport protein-related
At1g05190	-1.77	-2.99	-3.24	-3.67	-2.71	-2.88	ribosomal protein L6 family protein
At1g22430	-2.62	-1.48	-2.74		-4.64	-2.87	alcohol dehydrogenase
At1g56190	-1.79	-3.43	-4.03	-2.95	-2.10	-2.86	phosphoglycerate kinase
At5g02160	-2.05	-2.95	-4.04	-4.29	-0.97	-2.86	expressed protein
At2g34860	-1.97	-2.53	-3.28	-3.42	-3.05	-2.85	chaperone protein dnaJ-related
At3g56760	-0.51	-0.68	-3.97		-6.21	-2.84	calcium-dependent protein kinase
At1g09340	-2.48	-3.11	-1.02	-4.36	-3.24	-2.84	expressed protein
At2g32180	-2.40	-2.12	-1.69	-5.31	-2.65	-2.83	expressed protein
At5g37550	-1.27	-1.39	-2.49	-6.17		-2.83	expressed protein
At1g51630	-1.21	-1.54	-6.59	-3.49	-1.32	-2.83	expressed protein
At1g54780	-2.05	-2.06	-4.72	-2.96	-2.34	-2.82	thylakoid lumen 18.3 kDa protein
At5g02110	-1.18	-1.87	-2.57	-3.86	-4.59	-2.81	cyclin family protein
At2g07739	-1.36	-1.75	-2.64	-5.39		-2.78	expressed protein
At4g09400	-2.48	-3.23	-5.85	-2.25	-0.10	-2.78	hypothetical protein
At2g04039	-1.60	-2.08	-1.94	-3.54	-4.69	-2.77	expressed protein
At1g52230	-2.20	-2.65	-4.96	-2.76	-1.27	-2.77	photosystem I reaction center subunit VI
At4g01310	-2.06	-2.83	-1.95	-4.34	-2.66	-2.77	ribosomal protein L5 family protein
At4g13195	-0.16	-0.09	-5.45	-4.11	-4.01	-2.77	expressed protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At2g45180	-2.43	-3.24	-4.55	-3.42	-0.15	-2.76	protease inhibitor/LTP
At3g24430	-1.82	-1.68	-4.32	-3.15	-2.80	-2.75	expressed protein
At5g66190	-2.23	-1.71	-2.47	-4.21	-3.08	-2.74	ferredoxin NADP(+) reductase
At4g39770	-2.01	-1.49	-4.72			-2.74	trehalose-6-phosphate phosphatase
At5g66570	-2.04	-2.67	-4.33	-3.22	-1.42	-2.73	oxygen-evolving enhancer protein 1-1
At4g00370	-1.65	-0.86	-4.11	-3.93	-3.11	-2.73	sugar transporter family protein
At2g28605	-1.80	-2.26	-0.73	-5.54	-3.31	-2.73	expressed protein
At3g23610	-1.77	-3.90	-2.16	-3.32	-2.50	-2.73	dual specificity protein phosphatase
At3g56910	-1.94	-2.27	-3.04	-3.60	-2.77	-2.72	expressed protein
At2g23610	-1.92	-2.11	-3.53	-3.48	-2.57	-2.72	esterase
At5g07020	-2.03	-2.36		-3.39	-3.11	-2.72	proline-rich family protein
At3g48360	-1.39	-3.43	-3.33	-3.57	-1.87	-2.72	speckle-type POZ protein-related
At3g16370	-0.81	0.63	-4.02	-5.51	-3.84	-2.71	GDSL-motif lipase/hydrolase family protein
At2g42770			-0.74	-3.24	-4.15	-2.71	peroxisomal membrane 22 kDa family protein
At4g17560	-2.72	-2.25	-1.45	-4.13	-2.97	-2.70	ribosomal protein L19 family protein
At4g34190	-1.76	-2.49	-1.95	-4.07	-3.17	-2.69	stress enhanced protein 1
At4g38160	-1.37	-0.62	-4.08	-5.43	-1.94	-2.69	mitochondrial transcription termination factor-related
At1g60950	-1.65	-3.09	-1.68	-3.57	-3.39	-2.68	ferredoxin
At3g48720	-2.07	-1.79	-0.94	-5.29	-3.29	-2.68	transferase family protein
At1g56360	-2.03	-1.82	-4.18			-2.68	calcineurin-like phosphoesterase
At3g21055	-1.86	-3.12	-2.31	-3.68	-2.40	-2.68	photosystem II 5 kD protein
At2g28470		-2.43		-3.29	-2.30	-2.67	beta-galactosidase
At4g16410	-1.52	-2.08	-1.58	-4.02	-4.14	-2.67	expressed protein
At4g27657	-0.62	-1.51	-7.23	-3.45	-0.49	-2.66	expressed protein
At4g30880		-3.42	-1.41	-3.99	-1.80	-2.66	protease inhibitor/seed storage/LTP
At1g29910	-2.73	-2.00	-4.68	-3.88	0.02	-2.65	chlorophyll A-B binding protein 2
At5g64150	-1.66	-2.08	-3.82	-3.41	-2.28	-2.65	methylase family protein
At1g29440	-2.82	-2.33	-2.78			-2.64	auxin-responsive family protein
At2g21220	-3.00	-1.85	-3.05			-2.63	auxin-responsive protein
At3g19800	-1.49	-2.71	-1.28	-3.90	-3.76	-2.63	expressed protein
At4g10340	-2.56	-2.86	-3.01	-3.83	-0.87	-2.63	chlorophyll A-B binding protein
At4g20870	-1.74	-2.02	-2.33	-4.25	-2.78	-2.62	fatty acid hydroxylase
At1g60590	-1.88	-1.91	-0.72		-5.94	-2.61	polygalacturonase
At1g65510	-1.98	-2.33	-3.54	-3.17	-2.04	-2.61	expressed protein
At2g30790	-1.94	-2.41	-4.70	-2.71	-1.28	-2.61	photosystem II oxygen-evolving complex 23
At2g05620	-0.73	-1.33	-6.06	-1.80	-3.10	-2.61	expressed protein
At4g14440	-2.00	-2.24			-3.56	-2.60	enoyl-CoA hydratase/isomerase family protein
At1g03630	-2.42	-2.73	-2.27	-3.18	-2.40	-2.60	protochlorophyllide reductase C
At1g79915	-1.59	-1.10	-5.08			-2.59	hypothetical protein
At2g02590	-2.58	-3.32	-4.44	-1.72	-0.89	-2.59	expressed protein
At1g58520	-2.17	-1.11	-1.32		-5.74	-2.59	early-responsive to dehydration protein-related
At5g65620	-1.27	-1.16	-4.58		-3.33	-2.58	peptidase M3 family protein
At5g54270	-2.82	-2.39	-5.03	-2.48	-0.17	-2.58	chlorophyll A-B binding protein
At5g43750	-1.07	-1.46	-1.33	-4.89	-4.12	-2.57	expressed protein
At5g23010	-2.77	-3.39	-3.48		-0.64	-2.57	2-isopropylmalate synthase 3 (IMS3)
At1g29660	-2.29	-0.97	-0.12	-4.80	-4.60	-2.56	GDSL-motif lipase/hydrolase family protein
At3g59780	-1.72	-2.16	-1.55	-4.23	-3.11	-2.56	expressed protein
At1g05850	-1.79	-2.95	-2.82	-3.55	-1.66	-2.55	chitinase-like protein 1 (CTL1)
At1g61520	-2.31	-2.06	-5.45	-2.76	-0.20	-2.55	chlorophyll A-B binding protein
At1g22020	-1.54	-3.20	-2.82	-2.82	-2.35	-2.55	glycine hydroxymethyltransferase
At3g57040	-2.76	-2.30	-2.55			-2.54	two-component responsive regulator
At5g25610	-2.40	-2.76	-1.42	-3.98	-2.13	-2.54	dehydration-responsive protein
At1g67700	-1.78	-2.43	-2.41	-3.55	-2.45	-2.52	expressed protein
At5g36120	-2.40	-1.97	-1.79	-3.51	-2.95	-2.52	YGGT family protein
At5g54770	-2.17	-2.76	-1.88	-3.73	-2.06	-2.52	thiazole biosynthetic enzyme
At1g26590	-1.23	-1.68	-1.48		-5.66	-2.51	zinc finger (C2H2 type) family protein
At4g03520	-0.70	-1.33	-6.68	-2.25	-1.53	-2.50	thioredoxin M-type 2
At4g24810	-0.66	-0.94	-5.89			-2.50	ABC1 family protein
At1g56120	-1.93	-1.98	-3.57			-2.49	leucine-rich repeat family protein
At1g41830	-2.09	-2.39	-1.60	-4.01	-2.38	-2.49	multi-copper oxidase type I family protein
At1g18060	-2.41	-0.19	-1.44	-4.03	-4.38	-2.49	expressed protein
At4g23870	-2.49	-3.70	-3.53	-1.57	-1.16	-2.49	expressed protein
At4g39800	-2.04	-3.53	-1.39	-2.37	-3.11	-2.49	inositol-3-phosphate synthase isozyme 1
At5g40950	-1.38	-2.16	-2.60	-3.11	-3.20	-2.49	50S ribosomal protein L27
At2g43560	-1.58	-2.41	-2.59	-3.45	-2.38	-2.48	immunophilin
At4g20360	-1.48	-2.56	-2.98	-2.74	-2.58	-2.47	elongation factor Tu / EF-Tu (TUFA)
At5g44680	-1.46	-1.58			-4.32	-2.45	methyladenine glycosylase family protein
At2g30560	-1.09	-1.78	-6.60		-0.35	-2.45	glycine-rich protein
At5g18030	-2.67	-1.52	-2.85		-2.76	-2.45	auxin-responsive protein
At5g20630	-2.06	-3.55	-0.99	-2.71	-2.92	-2.44	germin-like protein (GER3)
At4g21240	-0.83	-2.94	-3.54			-2.44	F-box family protein
At5g11070	-1.22	-2.57	-3.31	-3.33	-1.74	-2.43	expressed protein
At5g37260	-0.74	-2.22	-1.10	-2.92	-5.18	-2.43	myb family transcription factor

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At5g06290	-1.58	-2.41	-1.58	-3.96	-2.60	-2.42	2-cys peroxiredoxin
At4g35090	-1.39	-1.31	-2.18	-2.55	-4.65	-2.41	catalase 2
At1g73655	-1.05	-2.91	-3.02	-3.08	-2.00	-2.41	immunophilin
At5g24150	-0.46	-0.21	-3.08	-3.52	-4.68	-2.39	squalene monooxygenase
At2g23590	-1.93	-2.55	-1.41		-3.65	-2.39	hydrolase, alpha/beta fold family protein
At1g26700	-2.75	-2.96	-1.89	-2.10	-2.22	-2.38	seven transmembrane MLO family protein
At4g04840	-0.72	-1.82	-1.31	-5.38	-2.67	-2.38	methionine sulfoxide reductase
At3g22210	-1.70	-2.13	-1.85	-2.92	-3.30	-2.38	expressed protein
At1g52220	-1.74	-2.27	-4.53	-2.82	-0.52	-2.38	expressed protein
At4g14970	-2.51	-2.19	-2.40			-2.37	hypothetical protein
At3g15354	-0.04	-0.34	-4.32	-2.30	-4.80	-2.36	WD-40 repeat family protein
At5g26742	-1.70	-1.30		-3.98	-2.48	-2.36	DEAD box RNA helicase
At1g77090	-1.78	-1.62	-2.01	-3.16	-3.19	-2.35	thylakoid lumenal 29.8 kDa protein
At5g08050	-1.43	-1.62	-0.97	-3.79	-3.97	-2.35	expressed protein
At2g33705	-2.18	-2.22	-2.41		-2.60	-2.35	F-box family protein
At3g55360	-0.40	-2.14	-4.36	-2.63	-2.22	-2.35	3-oxo-5-alpha-steroid 4-dehydrogenase
At3g54450	-0.27	-0.38	-5.40		-3.35	-2.35	POT family protein
At5g26000	-0.88	-3.84	-3.84	-1.89	-1.28	-2.35	glycosyl hydrolase family 1 protein
At1g29490	-2.06	-1.27	-1.49	-3.90	-3.01	-2.35	auxin-responsive family protein
At2g32650	-2.13	-2.29	-1.76	-3.75	-1.80	-2.35	expressed protein
At5g23310	-1.63	-1.64	-5.57	-1.54	-1.34	-2.34	superoxide dismutase
At3g44890	-1.34	-1.66	-1.89	-4.02	-2.82	-2.34	50S ribosomal protein L9
At3g02690	-1.36	-2.04	-1.58	-3.09	-3.64	-2.34	integral membrane family protein
At5g47380			-4.26	-1.55	-1.20	-2.34	expressed protein
At4g14100	-0.94	-2.32	-3.95	-2.38	-2.08	-2.33	expressed protein
At5g19220	-2.11	-1.79	-0.70	-3.66	-3.41	-2.33	glucose-1-phosphate adenylyltransferase
At2g24090	-1.87	-1.33	-0.69	-4.34	-3.42	-2.33	ribosomal protein L35 family protein
At3g01550	-2.57	-2.24	-1.02	-3.06	-2.76	-2.33	triose phosphate/phosphate translocator
At5g41610	-1.52	-2.09	-3.37			-2.32	cation/hydrogen exchanger
At2g23600	-1.61	-1.36	-1.25	-3.78	-3.62	-2.32	hydrolase, alpha/beta fold family protein
At3g21390	-1.28	-1.55		-2.82	-3.65	-2.32	mitochondrial substrate carrier family protein
At5g45930	-1.95	-2.31	-1.77	-3.05	-2.54	-2.32	magnesium-chelatase
At1g79560	-1.51	-0.95	-2.74	-3.54	-2.86	-2.32	FtsH protease
At1g55670	-2.36	-2.05	-3.45	-2.83	-0.90	-2.32	photosystem I reaction center subunit V
At3g55630	-1.85	-3.03		-0.58	-3.79	-2.31	dihydrofolate synthetase
At4g34760	-1.69	-0.49	-5.93	-1.98	-1.47	-2.31	auxin-responsive family protein
At1g55490	-2.11	-1.08	-0.87	-3.98	-3.51	-2.31	RuBisCO subunit binding-protein beta subunit
At2g33450	-1.51	-2.24	-1.90	-3.25	-2.63	-2.31	50S ribosomal protein L28
At2g26500	-0.90	-2.40	-3.37	-2.51	-2.36	-2.31	cytochrome b6f complex subunit (petM)
At1g10470	-0.33	-2.43	-1.65	-3.61	-3.51	-2.30	two-component responsive regulator
At4g12510	-1.81	-1.56	-6.04	-2.29	0.19	-2.30	protease inhibitor/seed storage/LTP
At4g13770	-1.96	-1.42	-2.85	-4.02	-1.27	-2.30	cytochrome P450 family protein
At5g44650	-1.40	-2.50	-2.05	-2.68	-2.89	-2.30	expressed protein
At3g01500	-1.56	-2.22	-1.10	-3.20	-3.42	-2.30	carbonic anhydrase 1
At5g19530	-2.88	-1.71	-2.30			-2.30	spermine/spermidine synthase family protein
At2g06906		-0.82	-2.23	-3.84		-2.30	hypothetical protein
At1g66910	-1.99	-1.99	-2.52	-2.99	-1.99	-2.30	protein kinase
At4g24780	-2.05	-1.69	-1.82	-2.97	-2.94	-2.29	pectate lyase family protein
At1g65295	-2.33	-2.96	-0.36		-3.51	-2.29	expressed protein
At5g59800	-2.10	-2.64	-3.15	-1.25		-2.29	methyl-CpG-binding domain-containing protein
At5g16200		-1.16	-3.26	-2.79	-1.93	-2.28	50S ribosomal protein-related
At5g65010	-1.44	-1.51	-0.49	-3.15	-4.82	-2.28	asparagine synthetase 2 (ASN2)
At5g03760	-1.15	-2.55	-1.72	-2.90	-3.09	-2.28	glycosyl transferase family 2 protein
At2g41820	-1.72	-3.16	-1.53	-2.36	-2.61	-2.28	leucine-rich repeat transmembrane protein kinase
At1g60550	-1.69	-2.80	-1.18	-2.99	-2.71	-2.27	naphthoate synthase
At4g37550	-2.05	-1.22	-2.03	-3.12	-2.95	-2.27	formamidase
At4g32260	-1.73	-1.49	-1.67	-3.58	-2.9	-2.27	ATP synthase
At4g17740	-0.66	-0.82	-5.98	-2.15	-1.74	-2.27	C-terminal processing protease
At2g15050	-1.62	-2.62	-1.58	-3.06	-2.46	-2.27	lipid transfer protein
At5g56500	-2.39	-2.48	-1.82		-2.37	-2.26	chaperonin
At3g16240	-0.02	-0.29	-4.31	-5.07	-1.61	-2.26	delta tonoplast integral protein
At1g43560	-1.35	-1.83	-2.33	-3.03	-2.73	-2.26	thioredoxin family protein
At1g29510	-2.90	-0.75	-3.37		-1.99	-2.25	auxin-responsive protein
At2g26900	-1.30	-1.03	-4.30	-2.90	-1.73	-2.25	bile acid:sodium symporter family protein
At4g23240	-1.45	-1.12	-4.18			-2.25	protein kinase family protein
At2g37220	-1.45	-2.69	-0.90	-3.59	-2.56	-2.24	29 kDa ribonucleoprotein
At3g61230	-1.19	-1.15	-4.37			-2.24	LIM domain-containing protein
At4g03280	-1.72	-1.87	-1.48	-3.44	-2.64	-2.23	cytochrome B6-F complex iron-sulfur subunit
At3g55330	-0.75	-2.53	-0.49	-3.89	-3.48	-2.23	photosystem II reaction center
At5g24105	-1.07	-1.70	-3.39	-3.24	-1.74	-2.23	expressed protein
At5g07870	-0.52	-1.75			-4.41	-2.23	transferase family protein
At5g46110	-1.71	-2.02	-3.13	-2.68	-1.58	-2.22	phosphate/triose-phosphate translocator
At1g01050	-0.75	-0.96	-6.20	-1.65	-1.56	-2.22	inorganic pyrophosphatase

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At2g03750	-1.59	-2.10	-2.00		-3.20	-2.22	sulfotransferase family protein
At3g26650	-0.62	-1.89	-0.49	-4.20	-3.89	-2.21	GAPA
At5g30510	-1.28	-2.14	-2.57	-2.69	-2.39	-2.21	30S ribosomal protein S1
At3g24770	-1.21	-1.96	-0.97	-3.61	-3.32	-2.21	CLE41
At3g63190	-2.37	-1.35	-1.17	-3.57	-2.59	-2.21	ribosome recycling factor
At4g17470	-1.30	-1.42	-1.85	-4.26	-2.21	-2.21	palmitoyl protein thioesterase family protein
At5g42760	-1.14		-0.61		-4.86	-2.20	O-methyltransferase
At1g68530	-1.69	-1.88	-2.29	-3.07	-2.07	-2.20	very-long-chain fatty acid condensing enzyme
At3g60890	-0.98	-1.51	-5.29	-2.32	-0.89	-2.20	hypothetical protein
At2g47930	-1.60	-1.47	-3.81		-1.90	-2.20	hydroxyproline-rich glycoprotein family protein
At4g26520	-1.81	-1.04			-3.74	-2.19	fructose-bisphosphate aldolase
At1g79000	-1.14	-1.55	-5.88	-1.94	-0.47	-2.19	p300/CBP acetyltransferase-related
At5g19940	-1.82	-1.54	-4.03	-2.37	-1.21	-2.19	plastid-lipid associated protein PAP-related
At3g55510	-1.95	-2.09	-2.53			-2.19	expressed protein
At5g37360	-1.20	-0.55	-5.39	-2.05	-1.76	-2.19	expressed protein
At5g11450	-1.79	-2.20	-0.66		-4.10	-2.19	oxygen-evolving complex-related
At2g42600	-1.76	-1.59	-1.43	-3.13	-3.01	-2.18	phosphoenolpyruvate carboxylase
At2g03350	-1.11	-1.33	-1.45	-5.99	-1.02	-2.18	expressed protein
At1g68010	-1.47	-1.44	-1.16	-3.65	-3.16	-2.17	glycerate dehydrogenase
At4g12440	-1.27	-0.94	-3.03	-2.94	-2.69	-2.17	adenine phosphoribosyltransferase
At2g38140	-0.89	-1.50	-1.84	-3.92	-2.71	-2.17	chloroplast 30S ribosomal protein S31
At5g13630	-2.43	-1.30	-1.69	-2.28	-3.14	-2.17	magnesium-chelatase
At4g27440	-2.12	-3.07		-3.15	-0.34	-2.17	protochlorophyllide reductase B
At2g01755	-0.94	-0.90	-4.90		-1.93	-2.17	hypothetical protein
At3g01480	-1.84	-1.56	-1.57	-3.19	-2.69	-2.17	peptidyl-prolyl cis-trans isomerase
At5g62370		-2.73	-0.44		-3.33	-2.17	pentatricopeptide (PPR) repeat-containing protein
At3g21890	-0.87	-1.39	-4.21			-2.16	zinc finger (B-box type) family protein
At5g17230	-1.06	-2.13	-1.72	-2.66	-3.19	-2.15	phytoene synthase (PSY)
At1g18090	-0.50	-1.68	-0.36	-6.05		-2.15	exonuclease
At2g36990	-1.18	-1.24	-1.44	-3.97	-2.90	-2.15	RNA polymerase sigma subunit SigF (sigF)
At1g32900	-2.22	-0.43	-0.41	-3.52	-4.15	-2.14	starch synthase
At1g29870	-1.29	-1.68	-3.46			-2.14	tRNA synthetase class II
At5g07200	-0.96	-2.26	-1.98	-2.71	-2.81	-2.14	gibberellin 20-oxidase
At1g60990	-1.49	-1.72	-1.07	-3.42	-3.01	-2.14	glycine cleavage T family protein
At5g01530	-1.64	-2.15	-3.42	-2.77	-0.73	-2.14	chlorophyll A-B binding protein
At3g27850	-1.31	-1.05	-1.10	-4.06	-3.18	-2.14	50S ribosomal protein L12-3
At2g39840	-1.93	-3.06	-3.26	-1.17	-1.24	-2.13	Ser/Thr phosphatase
At1g66820	-1.25	-2.05	-1.13	-3.44	-2.79	-2.13	glycine-rich protein
At3g61470	-2.05	-2.19	-2.49	-3.58	-0.34	-2.13	chlorophyll A-B binding protein
At2g29180	-1.78	-2.58	-1.49	-2.56	-2.21	-2.12	expressed protein
At1g48930	-1.46	-1.65	-3.27			-2.12	endo-1,4-beta-glucanase
At2g05070	-2.15	0.18	-5.10	-1.69	-1.85	-2.12	chlorophyll A-B binding protein
At5g38980	-1.91	-2.54	-2.28	-2.55	-1.31	-2.12	expressed protein
At4g24510	-1.60	-1.81			-2.94	-2.12	ecceriferum protein (CER2)
At5g65730	-1.85	-1.53	-2.54	-2.95	-1.70	-2.11	xyloglucan:xyloglucosyl transferase
At5g24630	-1.05	-1.23	-4.66		-1.50	-2.11	expressed protein
At1g22590	-0.48	-1.18	-2.65	-3.13	-3.12	-2.11	MADS-box family protein
At3g07690	-1.05	-2.75	-2.33	-2.24	-2.19	-2.11	glycerol-3-phosphate dehydrogenase
At1g06680	-1.58	-1.65	-2.23	-3.45	-1.63	-2.11	photosystem II oxygen-evolving complex 23
At4g02770	-1.39	-2.13	-4.60	-1.98	-0.43	-2.11	photosystem I reaction center subunit II
At2g34460	-1.04	-2.28	-1.20	-3.09	-2.90	-2.10	flavin reductase-related
At1g32080	-2.19	-1.14	-0.43	-3.15	-3.59	-2.10	membrane protein
At1g29070	-1.03	-1.20	-0.36	-4.42	-3.49	-2.10	ribosomal protein L34 family protein
At3g49720	-1.12	-2.41	-3.02	-2.63	-1.31	-2.10	expressed protein
At5g27400	-0.83	-1.03	-4.59	-2.35	-1.67	-2.10	expressed protein
At5g12250	-1.66	-2.09	-2.77	-3.17	-0.75	-2.09	tubulin beta-6 chain (TUB6)
At4g12800	-2.26	-1.64	-3.30	-2.95	-0.28	-2.09	photosystem I reaction center subunit XI
At4g08685	-0.94	-2.47	-2.09	-3.75	-1.16	-2.08	pollen Ole e 1 allergen and extensin family protein
At5g22640	-1.54	-2.25	-1.98		-2.52	-2.08	Membrane Occupation and Recognition Nexus
At1g11545	-2.91	-1.67	-1.17		-2.54	-2.07	xyloglucan:xyloglucosyl transferase
At2g28950	-2.41	-1.79	-0.30	-3.10	-2.77	-2.07	expansin (EXP6)
At1g13380	-0.98	-0.94	-4.03	-2.96	-1.43	-2.07	expressed protein
At5g06790	-0.93	-0.88	-1.15		-5.31	-2.07	expressed protein
At1g29700	-1.83	-1.94	-1.21	-2.92	-2.41	-2.06	expressed protein
At3g56940	-2.01	-1.86	-0.57	-2.87	-2.99	-2.06	dicarboxylate diiron protein
At2g18300	-2.27	-2.07	-1.13	-3.83	-0.99	-2.06	basic helix-loop-helix (bHLH) family protein
At4g27654	-0.68	-2.34	-3.37	-3.74	-0.17	-2.06	expressed protein
At2g47940	-1.44	-1.80	-1.53	-3.36	-2.16	-2.06	DegP2 protease (DEGP2)
At5g20140	-1.71	-1.73	-0.88	-3.56	-2.39	-2.06	SOUL heme-binding family protein
At1g30690	-1.16	-3.19	-2.07	-2.48	-1.37	-2.05	SEC14 cytosolic factor family protein
At3g42120	-1.82	-0.71	-3.63			-2.05	hypothetical protein
At5g59580	-0.54	-1.63	-3.99			-2.05	UDP-glucosyl transferase family protein
At1g56580	-1.35	-2.94	-0.88	-2.69	-2.39	-2.05	expressed protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At1g52510	-1.87	-1.18	-0.62	-3.50	-3.08	-2.05	hydrolase, alpha/beta fold family protein
At3g03840	-2.09	-1.85	-3.13		-1.12	-2.05	auxin-responsive protein
At2g34620	-1.08	-0.88	-4.19			-2.05	mitochondrial transcription termination factor-related
At1g01840	-0.70	-1.28	-0.96	-3.91	-3.38	-2.05	expressed protein
At1g49220	-2.32	-1.98	-1.85			-2.05	zinc finger (C3HC4-type RING finger) family protein
At5g17070	0.62	-0.82	-4.07	-3.92		-2.05	expressed protein
At5g51110	-0.20	-0.05	-2.22	-3.88	-3.88	-2.04	expressed protein
At3g05880	-0.92	-2.94	-3.10	-2.41	-0.84	-2.04	hydrophobic protein (RCI2A)
At2g26080	-1.48	-1.19	-1.54	-2.86	-3.14	-2.04	glycine dehydrogenase
At3g62910	-1.27	-1.34	-0.85	-3.66	-3.08	-2.04	peptide chain release factor
At1g30250	-1.34	-1.91	-0.41	-3.06	-3.47	-2.04	expressed protein
At1g63560	0.45	-1.67	-4.06		-2.86	-2.04	receptor-like protein kinase-related
At1g12160		-0.23	-3.44		-2.44	-2.03	flavin-containing monooxygenase family protein
At4g21210	-1.26	-1.60	-2.65	-2.57	-2.07	-2.03	expressed protein
At2g21490	-0.72	-2.64	-1.94	-2.36	-2.49	-2.03	dehydrin family protein
At4g12030	-0.25	-0.54	-3.94	-3.90	-1.51	-2.03	bile acid:sodium symporter family protein
At4g18810	-1.23	-0.25	-1.75	-3.12	-3.77	-2.03	expressed protein
At1g34720	-0.72	-1.47	-3.80		-2.11	-2.02	hypothetical protein
At2g24630	-1.56	-2.59	-1.92			-2.02	glycosyl transferase family 2 protein
At5g40940	-0.54		-1.74		-3.79	-2.02	hypothetical protein
At1g31920	-1.60	-2.47	-1.99			-2.02	pentatricopeptide (PPR) repeat-containing protein
At4g30330	-1.68	-0.91	-3.65		-1.83	-2.02	small nuclear ribonucleoprotein E
At5g13960	-1.88	-1.67	-2.30	-2.46	-1.77	-2.02	SET domain-containing protein (SUVH4)
At2g42975	-0.99	-0.79	-2.98	-1.44	-3.88	-2.01	expressed protein
At5g39270	-1.94	-1.82	-2.73		-1.56	-2.01	expansin (EXP22)
At5g64850	-1.12	-1.49	-0.60	-3.39	-3.46	-2.01	expressed protein
At5g22660	-1.92	-2.52	-3.53	-1.91	-0.18	-2.01	F-box family protein
At2g37630	-2.24	-2.03	-2.33	-1.45		-2.01	myb family transcription factor (MYB91)
At2g31470	-1.50	-1.65	-2.88			-2.01	F-box family protein
At2g16290	-1.63	-2.21	-2.19			-2.01	F-box family protein
At1g65080	-2.09	-3.05	-2.23	-1.67	-1.00	-2.01	OXA1 family protein
At1g32990	-2.08	-1.13	-1.68	-3.20	-1.95	-2.01	ribosomal protein L11 family protein
At5g35490	-1.95	-1.90	-0.09	-2.77	-3.33	-2.01	expressed protein
At3g63410	-2.18	-1.30	-0.06	-3.51	-2.97	-2.00	chloroplast inner envelope membrane protein
At5g21100		-0.62	-1.22	-2.92	-3.24	-2.00	L-ascorbate oxidase
At2g03550	-1.47	-1.84	-4.71	-1.36	-0.60	-2.00	expressed protein
At3g53850	-1.20	-0.32	-5.19		-1.27	-1.99	expressed protein
At1g74970	-1.34	-0.93	-1.35	-3.76	-2.59	-1.99	ribosomal protein S9 (RPS9)
At5g55450	-1.72	0.39	-2.46	-4.22	-1.95	-1.99	protease inhibitor/seed storage/LTP
At1g05690	-1.65	-1.39	-2.94			-1.99	TAZ zinc finger family protein
At4g33220	-1.78	-2.35	-1.46		-2.37	-1.99	pectinesterase family protein
At1g23310	-1.05	-2.41	-2.45	-1.39	-2.66	-1.99	glutamate:glyoxylate aminotransferase 1
At1g62750	-1.03	-1.39	-2.05	-2.38	-3.11	-1.99	elongation factor Tu family protein
At2g17695	-1.63	-2.04	-1.67	-2.24	-2.36	-1.99	expressed protein
At2g41950	-0.42	-0.99	-3.10	-2.97	-2.46	-1.99	expressed protein
At4g03979		-0.15	-1.35		-4.46	-1.99	hypothetical protein
At2g24650	-0.83	-0.28	-4.08		-2.75	-1.99	transcriptional factor B3 family protein
At3g56090	-0.86	-0.86	-4.89	-1.79	-1.53	-1.98	ferritin
At3g09640	-1.23	-1.45	-2.28	-2.92	-2.04	-1.98	L-ascorbate peroxidase 1b (APX1b)
At3g27160	-1.44		-0.73	-3.16	-2.60	-1.98	ribosomal protein S21 family protein
At1g80640	-1.86	-0.64	-3.92	-2.07	-1.41	-1.98	protein kinase family protein
At1g27480	-1.09	-2.51	-1.11	-1.00	-4.18	-1.98	lecithin:cholesterol acyltransferase family protein
At3g54890	-2.05	-2.27	-2.80	-2.64	-0.10	-1.97	chlorophyll A-B binding protein
At3g19720	-1.85	-0.39	-2.91	-2.45	-2.23	-1.97	dynamin family protein
At1g45010	-2.27	-2.31	-1.43	-1.61	-2.21	-1.96	expressed protein
At1g27590	-1.03	-1.96	-2.89			-1.96	expressed protein
At2g29980	-1.85	-0.63	-0.56	-4.00	-2.77	-1.96	omega-3 fatty acid desaturase
At1g75460	-1.31	-1.26	-2.47	-2.36	-2.34	-1.95	ATP-dependent protease
At3g12230	-2.22	-0.52	-2.40	-0.40	-4.21	-1.95	serine carboxypeptidase S10 family protein
At3g10405	-0.70	-1.67	-2.29	-3.27	-1.79	-1.95	expressed protein
At5g55620	-1.63	-1.41	-0.16	-5.46	-1.08	-1.95	expressed protein
At3g59730	-1.16	-1.48	-2.78	-3.47	-0.82	-1.94	receptor lectin kinase
At4g35100	-1.22	-1.58	-1.95	-2.59	-2.38	-1.94	plasma membrane intrinsic protein
At5g09220	-2.14	-2.52	-1.46		-1.65	-1.94	amino acid permease 2 (AAP2)
At2g17090		-1.85	-3.75	-0.22		-1.94	protein kinase family protein
At2g16280	-0.25	-1.53	-4.97	-0.76	-2.19	-1.94	very-long-chain fatty acid condensing enzyme
At5g14740	-1.92	-1.74	-0.88	-1.86	-3.29	-1.94	carbonic anhydrase 2
At4g36540	-1.58	-1.11	-1.18	-3.91	-1.91	-1.94	basic helix-loop-helix (bHLH) family protein
At1g04240	-2.52	-1.78	-1.19	-2.54	-1.64	-1.93	auxin-responsive protein
At2g40610	-1.50	-1.89	-2.59	-1.99	-1.68	-1.93	expansin (EXP8)
At1g46264	-2.55	-0.68	-2.56			-1.93	heat shock transcription factor family protein
At4g38970	-1.00	-0.80	-0.70	-3.76	-3.37	-1.93	fructose-bisphosphate aldolase
At1g53350	-1.05	-0.73	-1.71	-3.44	-2.69	-1.92	disease resistance protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At2g34680	-1.02	-1.49	-2.41	-2.86	-1.83	-1.92	leucine-rich repeat family protein
At1g03870	-1.54	-1.33	-1.41	-3.27	-2.06	-1.92	fasciclin-like arabinogalactan-protein
At3g62410	-0.96	-2.22	-0.36	-2.63	-3.39	-1.91	CP12 domain-containing protein
At2g45470	-1.88	-1.63	-3.51	-2.42	-0.12	-1.91	fasciclin-like arabinogalactan-protein
At2g38730	-1.48	-2.77	-2.70	-1.17	-1.44	-1.91	peptidyl-prolyl cis-trans isomerase
At4g34800	-0.18	-2.71			-2.84	-1.91	auxin-responsive family protein
At2g35370	-1.96	-1.85	-0.06	-2.61	-3.07	-1.91	glycine cleavage system H protein 1
At4g22890	-1.22	-1.68	-1.30	-2.55	-2.78	-1.91	expressed protein
At5g19850	-0.69	-0.2	-2.98	-1.58	-4.08	-1.91	hydrolase, alpha/beta fold family protein
At3g05890	-2.87	-1.17	-3.26	-1.91	-0.30	-1.90	hydrophobic protein (RCI2B)
At1g71865	-0.88	-1.59	-5.39	-0.67	-0.97	-1.90	expressed protein
At3g26570	-0.66	-1.16	-1.91	-1.72	-4.07	-1.90	phosphate transporter family protein
At1g49975	-1.26	-0.94	-1.58	-4.02	-1.70	-1.90	expressed protein
At5g02840	-0.34	-0.35	-3.30	-2.56	-2.94	-1.90	myb family transcription factor
At1g69530	-1.72	-1.42	-1.30	-2.42	-2.63	-1.90	expansin (EXP1)
At5g63420	-1.08	-2.22	-2.32	-2.13	-1.73	-1.90	metallo-beta-lactamase family protein
At2g38360	-0.39	-2.11	-4.45	-1.50	-1.02	-1.89	prenylated rab acceptor (PRA1) family protein
At4g25100	-1.10	-2.08	-2.96	-1.93	-1.39	-1.89	superoxide dismutase
At3g49500	-1.60	-1.57	-2.50			-1.89	RNA-dependent RNA polymerase (SDE1)
At4g27520	-1.42	-1.36	-2.76	-2.56	-1.35	-1.89	plastocyanin-like domain-containing protein
At5g24710	-1.25	-2.97	-1.31	-1.92	-1.99	-1.89	WD-40 repeat family protein
At5g64580	-1.05	-1.18		-2.70	-2.61	-1.88	AAA-type ATPase family protein
At3g11680	-1.96	-1.45	-2.24			-1.88	expressed protein
At1g74930	-0.61	-1.98	-2.66	-4.49	0.33	-1.88	AP2 domain-containing transcription factor
At4g12150	-2.06	-2.74	-2.24		-0.50	-1.88	zinc finger (C3HC4-type RING finger) family protein
At3g27690	-1.63	-0.68	-2.44	-2.69	-1.96	-1.88	chlorophyll A-B binding protein
At1g68590	-1.72	-1.15	-0.82	-2.83	-2.88	-1.88	plastid-specific 30S ribosomal protein 3
At4g18740	-1.77	-1.01	-0.99	-2.61	-3.02	-1.88	expressed protein
At4g28700	-1.52	-2.10	-1.42		-2.48	-1.88	ammonium transporter
At1g27210	-1.22	-1.72	-2.70			-1.88	expressed protein
At1g27950	-1.45	-1.53	-0.16	-3.26	-2.98	-1.88	lipid transfer protein-related
At3g05150	-0.91	-0.35	-4.37			-1.87	sugar transporter family protein
At5g26950	-1.86	-1.74	-0.75	-2.61	-2.40	-1.87	MADS-box family protein
At1g78020	-0.98	-1.73	-2.05	-1.92	-2.67	-1.87	senescence-associated protein-related
At5g52930	-1.33	-0.81	-3.46			-1.87	expressed protein
At3g01440	-1.12	-0.73	-0.54	-3.47	-3.46	-1.87	oxygen evolving enhancer 3
At1g35170	-0.55	0.06	-3.26	-2.54	-3.03	-1.86	expressed protein
At2g20930	-1.08	-1.95	-0.66	-2.71	-2.92	-1.86	expressed protein
At1g10030	-1.01	-0.88	-2.59	-2.43	-2.40	-1.86	integral membrane family protein
At2g34430	-1.53	-2.67	-2.77	-3.30	0.97	-1.86	chlorophyll A-B binding protein
At2g25080	-1.02	-0.96	-3.18	-2.58	-1.56	-1.86	phospholipid hydroperoxide glutathione peroxidase
At3g13100	-2.70	-3.00	-3.82	0.13	0.12	-1.85	ABC transporter family protein
At3g57210	-1.59	-2.44	-0.40	-3.08	-1.70	-1.84	hypothetical protein
At5g17300	-1.14	-0.66	-0.96	-2.69	-3.75	-1.84	myb family transcription factor
At5g41900	-2.13	-1.60	-1.79			-1.84	hydrolase, alpha/beta fold family protein
At4g02920	-1.56	-1.48	-0.54	-2.34	-3.30	-1.84	expressed protein
At1g73600	-1.16	-0.72	-2.83	-2.50	-2.00	-1.84	phosphoethanolamine N-methyltransferase 3
At2g21385	-2.08	-0.74	-1.47		-3.06	-1.84	expressed protein
At1g13820	-1.72	-0.59			-3.19	-1.83	hydrolase, alpha/beta fold family protein
At3g52320	-1.70	-2.56	-2.16	-0.84	-1.90	-1.83	F-box family protein
At5g57920	-1.11	-2.04	-1.11	-2.65	-2.24	-1.83	plastocyanin-like domain-containing protein
At2g39670	-1.34	-1.26	-0.54	-3.63	-2.38	-1.83	radical SAM domain-containing protein
At3g11550	-2.44	-2.21	-0.93	-1.75		-1.83	integral membrane family protein
At5g57170	-0.17	-0.86	-1.27	-4.31	-2.54	-1.83	MIF family protein
At1g64510	-1.43	-1.69	-1.11	-2.62	-2.28	-1.82	ribosomal protein S6 family protein
At5g12150	-1.11	-1.95	-0.98	-3.17	-1.91	-1.82	pleckstrin homology (PH) domain-containing protein
At1g70410	-1.53	-1.85	-0.48	-3.03	-2.22	-1.82	carbonic anhydrase
At5g65100	-1.06	-1.17	-0.26	-4.60	-2.00	-1.82	ethylene insensitive 3 family protein
At4g34830	-1.72	-1.15	-0.19	-2.06	-3.96	-1.82	pentatricopeptide (PPR) repeat-containing protein
At4g21870	-1.77	-1.08	-1.90	-2.51		-1.82	Heat shock protein 18.2
At1g10960	-0.97	-0.81	-1.17	-2.69	-3.43	-1.82	ferredoxin, chloroplast
At2g26870	-0.91	-0.43	-4.55	-0.66	-2.53	-1.81	phosphoesterase family protein
At5g51820		-0.59	-0.64	-3.62	-2.40	-1.81	phosphoglucomutase
At1g66430	-1.64	-1.94	-1.42		-2.25	-1.81	pfkB-type carbohydrate kinase family protein
At3g56480	-1.30	-1.78		-2.40	-1.76	-1.81	myosin heavy chain-related
At1g20795	-1.58	-2.89	-0.95			-1.81	F-box family protein
At4g12420	-2.02	-1.57	-1.53	-3.06	-0.86	-1.81	multi-copper oxidase
At1g12580	-1.64	-1.99	-1.34	-2.18	-1.87	-1.81	protein kinase family protein
At2g31730	-1.51	-1.50	-2.16	-0.10	-3.75	-1.80	ethylene-responsive protein
At5g26780	-1.63	-2.37	-1.93	-1.64	-1.42	-1.80	glycine hydroxymethyltransferase
At3g27050	-0.55	-0.61	-2.66	-1.64	-3.52	-1.80	expressed protein
At5g48790			-0.88	-2.47	-2.03	-1.79	expressed protein
At1g18620	-1.10	-1.44	-4.17	-1.28	-0.98	-1.79	expressed protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At3g29280	-0.82	-1.26	-1.54	-2.70	-2.64	-1.79	expressed protein
At3g08920	-1.92	-1.01	-1.47		-2.76	-1.79	rhodanese-like domain-containing protein
At3g17620	-2.00	-3.13	-0.23			-1.79	F-box family protein
At2g16440	-0.62	-1.65	-1.57	-1.96	-3.13	-1.78	DNA replication licensing factor
At1g11720	-1.05	-1.39	-1.12	-2.01	-3.34	-1.78	starch synthase
At3g05780	-1.62	-1.87	-1.85			-1.78	Lon protease
At1g72630	-1.53	-1.64	-2.41	-1.72	-1.60	-1.78	expressed protein
At2g21970	-0.31	-1.20	-3.90	-1.25	-2.25	-1.78	stress enhanced protein 2
At1g53300	-1.84	-1.25	-1.03	-2.97	-1.8	-1.78	thioredoxin family protein
At3g25660	-1.55	-1.35	-1.01	-3.05	-1.92	-1.78	glutamyl-tRNA(Gln) amidotransferase
At2g39795	-0.54	-2.87	-1.38	-2.55	-1.53	-1.77	mitochondrial glycoprotein family protein
At3g47860	-1.11	-2.09	-0.23	-4.17	-1.23	-1.77	apolipoprotein D-related
At1g09750	-0.61	-1.61	-0.97	-4.01	-1.62	-1.76	chloroplast nucleoid DNA-binding protein-related
At1g60000	-1.26	-1.04	-1.79	-2.10	-2.63	-1.76	29 kDa ribonucleoprotein
At1g77490	-0.42	-0.98	-2.86	-2.85	-1.71	-1.76	L-ascorbate peroxidase
At4g23290	-1.64	-0.76	-2.24	-0.57	-3.60	-1.76	protein kinase family protein
At2g47400	-0.26	-1.90	-3.78	-1.54	-1.32	-1.76	CP12 domain-containing protein
At5g60890	-0.69	-1.58	-1.69		-3.08	-1.76	receptor-like protein kinase (ATR1) (MYB34)
At1g10370	-1.31	-2.33	-1.48	-2.04	-1.60	-1.75	glutathione S-transferase
At3g48040	-1.55	-1.41	-1.94		-2.10	-1.75	Rac-like GTP-binding protein (ARAC8)
At1g15820	-1.81	-2.05	-2.11	-2.19	-0.56	-1.75	chlorophyll A-B binding protein
At1g33700	-1.55	-1.72	-1.96			-1.75	expressed protein
At3g61210	-1.19	-1.62	-2.71	-2.83	-0.36	-1.74	embryo-abundant protein-related
At3g14420	0.09	-0.08	-2.00	-3.19	-3.53	-1.74	(S)-2-hydroxy-acid oxidase
At1g51080	-0.83	-1.10	-1.98	-3.06		-1.74	expressed protein
At1g49760	-0.97	-1.23	-0.61	-2.22	-3.67	-1.74	polyadenylate-binding protein
At4g01150	-0.86	-1.78	-0.77	-2.96	-2.33	-1.74	expressed protein
At2g27385	-1.09	-1.16	-1.08	-3.45	-1.92	-1.74	expressed protein
At4g35290	-0.71	-1.6	-2.89			-1.74	glutamate receptor family protein
At2g31100	-2.33	-2.15	-2.69	-1.77	0.27	-1.73	lipase
At1g72645	-1.27	-0.72	-1.06		-3.89	-1.73	expressed protein
At4g30810	-0.59	-1.29	-1.41	-2.05	-3.33	-1.73	serine carboxypeptidase S10 family protein
At3g55440	-0.58	-1.87	-4.17	-1.40	-0.63	-1.73	triosephosphate isomerase
At4g00490	-1.65	-1.59	-0.09	-3.09	-2.22	-1.73	beta-amylase
At1g70370	-0.85	-0.94	-0.40	-3.33	-3.10	-1.73	BURP domain-containing protein
At1g44970	-1.50	-2.41	-0.80		-2.18	-1.72	peroxidase
At2g13610		-0.13	-1.89		-3.15	-1.72	ABC transporter family protein
At1g78630	-0.98	-1.01	-0.66	-3.44	-2.51	-1.72	ribosomal protein L13 family protein
At1g37130	-0.71	-1.32	-2.80	-0.97	-2.80	-1.72	nitrate reductase 2 (NR2)
At3g06070	-0.37	-1.50	-1.27	-2.56	-2.89	-1.72	expressed protein
At3g08740	-1.52	-0.46	-0.69	-2.86	-3.05	-1.72	elongation factor P (EF-P) family protein
At5g64130	-0.93	-2.37	-3.67	-1.13	-0.47	-1.71	expressed protein
At1g75030	-1.28	-1.69	-3.13	-1.11	-1.36	-1.71	pathogenesis-related thaumatin family protein
At1g29720	-1.24	-0.90	-0.21	-3.59	-2.63	-1.71	protein kinase family protein
At4g29060	-1.66	-1.56	-2.18	-1.27	-1.90	-1.71	elongation factor Ts family protein
At5g12860	-1.42	-1.48	-1.31	-2.07	-2.29	-1.71	oxoglutarate/malate translocator
At2g19650	-0.65		-0.87	-2.57	-2.75	-1.71	DC1 domain-containing protein
At2g26730	-1.65	-1.91	-1.11	-2.11	-1.76	-1.71	leucine-rich repeat transmembrane protein kinase
At5g59500	-0.47	-0.07	-3.51	-2.58	-1.91	-1.71	expressed protein
At1g68520	-1.25	-2.55		-1.85	-1.18	-1.70	zinc finger (B-box type) family protein
At5g55490	-0.19		-2.64	-3.23	-0.75	-1.70	expressed protein
At1g16170	-1.66	0.370	-0.74	-3.45	-3.04	-1.70	expressed protein
At4g37230	-1.32	-2.22	-0.41	-2.49	-2.07	-1.70	oxygen-evolving enhancer protein
At3g56010	-1.05	-1.73	-1.38	-2.12	-2.22	-1.70	expressed protein
At5g57100	-1.40	-1.13		-2.53	-1.74	-1.70	transporter-related
At3g55680	-1.66	-1.75	-3.40	-1.67	-0.01	-1.70	invertase/pectin methylesterase inhibitor family protein
At2g44940	-1.23	-1.79	-2.10	-0.66	-2.68	-1.69	AP2 domain-containing transcription factor
At5g61130	-0.90	-0.65			-3.52	-1.69	glycosyl hydrolase family protein 17
At2g23380	-0.47	-0.72	-1.65		-3.92	-1.69	curly leaf protein
At2g47590	-0.24	-0.97	-0.34	-4.32	-2.57	-1.69	photolyase/blue light photoreceptor
At4g32760	-0.62	-1.97	-0.49	-2.70	-2.66	-1.69	VHS domain-containing protein
At5g58300	-2.05	-1.73	-2.76	-1.34	-0.55	-1.69	leucine-rich repeat transmembrane protein kinase
At1g18780	-1.74	-1.26	-2.63	-1.11		-1.68	zinc finger (C3HC4-type RING finger) family protein
At5g62720	-0.74	-0.51	-1.64	-2.68	-2.85	-1.68	integral membrane HPP family protein
At3g62960	-1.82	-1.99	-0.65		-2.27	-1.68	glutaredoxin family protein
At4g02680	-1.02	-1.99	-1.30	-2.17	-1.93	-1.68	tetratricopeptide repeat
At4g26850	-0.68	-1.25	-2.92	-1.05	-2.51	-1.68	expressed protein
At4g39010	-0.10	-0.33	-1.01	-3.97	-3.00	-1.68	glycosyl hydrolase family 9 protein
At4g15056	-1.03	-0.39	-2.11	-3.19		-1.68	hypothetical protein
At4g26040	-1.72	-1.75	-2.94	-0.31		-1.68	hypothetical protein
At1g19920	-0.16	-0.44	-4.19	-1.54	-2.06	-1.68	sulfate adenylyltransferase 2
At2g24020	-0.98	-1.45	-1.73	-2.28	-1.93	-1.68	expressed protein
At1g44446	-1.05	-0.71	-1.01	-2.03	-3.58	-1.68	chlorophyll a oxygenase (CAO)

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At1g75350	-1.30	-0.39	-3.38	-1.63	-1.68	-1.68	ribosomal protein L31 family protein
At1g50120	-0.64	-1.01	-4.43	-1.07	-1.22	-1.67	expressed protein
At1g11860	-1.33	-0.50	-0.62	-2.65	-3.28	-1.67	aminomethyltransferase
At4g11190	-1.75	-1.13	-2.14			-1.67	disease resistance-responsive family protein
At2g33180	-1.27	-0.73	-0.94	-2.50	-2.91	-1.67	expressed protein
At1g32220	-1.15	-0.29	-0.99	-3.20	-2.72	-1.67	expressed protein
At3g52150	-1.82	-1.30	0.52	-3.14	-2.58	-1.66	RNA recognition motif (RRM)-containing protein
At1g76620	-1.07	-1.04	-1.04	-3.84	-1.33	-1.66	expressed protein
At1g27400	-0.98	-1.96		-2.37	-1.34	-1.66	60S ribosomal protein L17
At2g36835	-0.45	-1.63	-1.66	-1.93	-2.64	-1.66	expressed protein
At1g33670	-0.06	-1.57	-3.35			-1.66	leucine-rich repeat family protein
At2g42740	-0.87	-0.39	-4.46	-1.67	-0.90	-1.66	60S ribosomal protein L11
At4g15140	-1.08	-0.73	-4.76	-1.12	-0.61	-1.66	expressed protein
At2g21650	-0.81	-0.29	-3.33	-2.17		-1.65	myb family transcription factor
At2g35970	-0.94	-1.86	-1.65	-2.25	-1.56	-1.65	harpin-induced family protein
At5g64860	-0.18	-2.76	-0.37	-1.86	-3.09	-1.65	4-alpha-glucanotransferase
At3g25850	-1.56	-1.37	-2.60	-2.09	-0.62	-1.65	DC1 domain-containing protein
At2g35470	-0.74	-0.55	-3.37	-3.03	-0.55	-1.65	expressed protein
At2g02130	-0.88	-1.42	-1.79	-2.75	-1.41	-1.65	plant defensin-fusion protein
At1g02010	-1.42	-2.68	-1.73	-0.40	-1.99	-1.65	cytokinesis-related Sec1 protein
At4g18030	-1.21	-1.93	-2.13	-2.04	-0.92	-1.64	dehydration-responsive family protein
At4g01935	-1.00	-1.52			-2.41	-1.64	expressed protein
At1g70940	-1.82	-1.15	-2.68	-1.55	-1.02	-1.64	auxin transport protein
At3g60210	-0.65	-1.87	-2.04	-2.09	-1.56	-1.64	chloroplast chaperonin 10
At5g19190	-1.48	-0.64	-1.59	-2.34	-2.17	-1.64	expressed protein
At1g48600	-1.34	-1.25	-1.64	-2.38	-1.60	-1.64	phosphoethanolamine N-methyltransferase 2
At3g43720	-0.75	-0.77	-0.23	-3.52	-2.93	-1.64	protease inhibitor/seed storage/LTP
At5g33715	-1.07	-1.29	-0.18	-3.38	-2.28	-1.64	hypothetical protein
At3g49220	-1.38	-1.20	-2.17	-2.07	-1.37	-1.64	pectinesterase family protein
At1g70550	-2.27	-1.04	-1.60			-1.64	expressed protein
At1g50900	-0.49	-1.41	-0.47	-3.74	-2.06	-1.64	expressed protein
At2g22230	-0.79	-1.13		-2.65	-1.97	-1.63	beta-hydroxyacyl-ACP dehydratase
At3g61080	-1.57	-0.81	-1.67	-2.16	-1.95	-1.63	fructosamine kinase family protein
At5g53170	-0.76	-1.22	-2.22	-2.25	-1.72	-1.63	FtsH protease
At4g34820	-0.47	-1.95	-0.51	-2.46	-2.77	-1.63	expressed protein
At1g54926	-1.30	-0.97	-0.21	-3.26	-2.42	-1.63	hypothetical protein
At3g05410	-1.45	-1.23	-0.75		-3.09	-1.63	expressed protein
At5g38510	-1.34	-1.20	-0.79	-2.87	-1.93	-1.63	rhomboid family protein
At3g02830	-0.98	-1.81	0.27	-1.79	-3.81	-1.62	zinc finger (CCCH-type) family protein
At5g57040	-0.86	-1.00	-3.19	-1.87	-1.16	-1.62	lactoylglutathione lyase family protein
At5g10480	-0.92	-1.80	-1.25	-2.21	-1.90	-1.62	protein tyrosine phosphatase-like protein
At1g04680	-0.65	-1.11	-0.58		-4.12	-1.62	pectate lyase family protein
At3g51950	-1.18	-0.60	-2.08	-1.25	-2.96	-1.61	zinc finger (CCCH-type) family protein
At3g56650	-1.54	-1.07	-0.22	-3.29	-1.95	-1.61	thylakoid lumenal 20 kDa protein
At5g14200	-1.21	-2.10	-1.50	-1.71	-1.55	-1.61	3-isopropylmalate dehydrogenase
At2g32560	-0.11	-0.22	-2.55	-2.59	-2.60	-1.61	F-box family protein
At2g30420	-2.45	-1.61	-0.78			-1.61	myb family transcription factor
At3g26540	-2.36	-2.51	-0.59	-1.83	-0.77	-1.61	pentatricopeptide repeat-containing protein
At2g38350	-2.17	-1.15	-1.51			-1.61	hypothetical protein
At3g27840	-1.24	-0.99	-0.83	-2.97	-2.02	-1.61	50S ribosomal protein L12-2
At5g06265	-0.63	-1.23	-2.71	-2.41	-1.05	-1.61	hyaluronan mediated motility receptor-related
At1g26945	-2.22	-0.88	-0.63	-4.19	-0.11	-1.61	expressed protein
At3g53450	-1.19	-2.27	-1.24		-1.72	-1.60	hypothetical protein
At1g61095	-0.14	-0.67	-3.54		-2.07	-1.60	expressed protein
At3g02110	-1.43		-1.54		-1.84	-1.60	serine carboxypeptidase S10 family protein
At5g17870	-0.60	-1.03	-0.59	-3.10	-2.69	-1.60	plastid-specific ribosomal protein-related
At1g66145	-1.36	-1.48	-2.49	-2.07	-0.60	-1.60	CLE18
At4g12560	-0.22	-0.63	-3.87	-1.38	-1.90	-1.60	F-box family protein
At3g08940	-1.65	-0.95	-2.53	-2.09	-0.77	-1.60	chlorophyll A-B binding protein
At3g02060	-0.87	-0.68		-2.98	-1.85	-1.60	DEAD/DEAH box helicase
At1g78450	-1.56	-1.45	-1.78			-1.60	SOUL heme-binding family protein
At5g44530	-0.87	-0.79	-0.13	-3.70	-2.49	-1.59	subtilase family protein
At5g16540	-0.99	-1.50	-0.80	-2.27	-2.41	-1.59	zinc finger (CCCH-type) family protein
At4g15510	-0.60	-0.57	-2.04	-2.77	-2.00	-1.59	photosystem II reaction center
At1g79040	-0.96	-1.41	-1.54	-3.22	-0.82	-1.59	photosystem II 10 kDa polypeptide
At3g52500	-1.37	-2.93	-1.83	-2.23	0.40	-1.59	aspartyl protease family protein
At5g16140	-0.82	-0.90	-2.18		-2.46	-1.59	peptidyl-tRNA hydrolase family protein
At4g04350	-1.29	-0.49		-2.41	-2.16	-1.59	leucyl-tRNA synthetase
At5g39910	-1.74	-2.57	-1.44	-1.46	-0.73	-1.59	glycoside hydrolase family 28 protein
At4g31040	-1.39	-1.16		-2.51	-1.30	-1.59	proton extrusion protein-related
At2g42710	-1.13	-2.23	-2.23	-1.03	-1.32	-1.59	ribosomal protein L1 family protein
At4g07310	-0.86	-0.01	-0.74	-3.30	-3.01	-1.58	hypothetical protein
At4g26370	-0.90	-0.82	-0.82	-3.20	-2.18	-1.58	antitermination NusB domain-containing protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At3g03830	-1.71	-1.09	-3.40	-0.51	-1.21	-1.58	auxin-responsive protein
At5g45940	-0.70	-1.95	-1.86		-1.82	-1.58	MutT/nudix family protein
At3g26470	-1.59	-1.12	-3.05	-0.91	-1.22	-1.58	expressed protein
At3g44910	-1.29	-0.65	-1.12	-2.44	-2.39	-1.58	cation/hydrogen exchanger
At3g01510	-0.64	-0.07		-4.00		-1.57	5'-AMP-activated protein kinase
At5g48890	-0.02	-0.19	-3.18	-2.64	-1.84	-1.57	hypothetical protein
At4g15640	-1.19	-1.09	-2.84	-2.12	-0.62	-1.57	expressed protein
At1g05385	-1.07	-1.79	-0.52	-1.92	-2.55	-1.57	photosystem II 11 kDa protein-related
At2g41310	-1.94	-1.21	-0.41		-2.70	-1.57	two-component responsive regulator
At4g12830	-1.28	-1.74	-0.34	-2.92		-1.57	hydrolase, alpha/beta fold family protein
At4g17090	-0.36	-2.43	-0.94	-1.50	-2.59	-1.57	beta-amylase (CT-BMY)
At1g05030	-1.33	-1.75	-2.42	-1.88	-0.45	-1.57	hexose transporter
At3g26960	-2.01	-1.74	-0.52	-2.06	-1.50	-1.57	expressed protein
At3g45160	-0.55	-1.01	-3.76	-1.14	-1.36	-1.56	expressed protein
At3g27060			-1.30	-1.51	-1.89	-1.56	ribonucleoside-diphosphate reductase small chain
At3g55040	-1.55	-0.91	-0.35	-2.98	-2.02	-1.56	In2-1 protein
At1g65260	-0.88	-1.35	-2.45	-1.69	-1.44	-1.56	PspA/IM30 family protein
At4g38495		-1.50	-2.58	-0.97	-2.75	-1.56	expressed protein
At2g40100	-0.82	-0.37	-1.06	-2.10	-3.45	-1.56	chlorophyll A-B binding protein
At4g29830	-0.21	-0.43		-1.88	-3.72	-1.56	transducin family protein
At4g27910	-0.42	-0.24	-2.85	-1.18	-3.10	-1.56	PHD finger protein-related
At4g10770	-1.13	-1.16	-0.81	-2.56	-2.13	-1.56	oligopeptide transporter OPT family protein
At5g57800	-1.00	-1.35	-2.43	-1.68	-1.32	-1.56	CER1 protein
At4g23350	-1.35	-1.39	-1.92			-1.55	expressed protein
At4g34290	-1.41	0.11	-0.17	-3.75	-2.55	-1.55	SWIB complex BAF60b domain-containing protein
At4g23260	-0.76	-2.09	-2.08	-1.08	-1.76	-1.55	protein kinase family protein
At1g31230	-1.87	-1.45			-1.34	-1.55	bifunctional aspartate kinase
At4g04570	-1.00	-0.64	-0.69	-1.83	-3.59	-1.55	protein kinase family protein
At3g60750	-0.77	-1.58	-1.00	-1.91	-2.48	-1.55	transketolase
At5g27560	-1.46	-0.72	-0.43	-2.94	-2.18	-1.55	expressed protein
At1g24400	-0.79	-0.87	-2.97			-1.54	lysine and histidine specific transporter
At1g44830	-0.10	-1.47	-3.07			-1.54	AP2 domain-containing transcription factor
At3g26070	-1.22	-1.61	-0.85	-2.31	-1.72	-1.54	plastid-lipid associated protein PAP
At1g32380	-0.17	-0.24	-4.14	-1.75	-1.40	-1.54	ribose-phosphate pyrophosphokinase 2
At5g54600	-0.71	-1.35	-1.73	-2.09	-1.81	-1.54	50S ribosomal protein L24
At2g38025	-0.62	-1.18	-3.76	-0.97	-1.16	-1.54	expressed protein
At4g21960	-1.34	-2.38	-2.42	-1.58	0.04	-1.54	peroxidase 42 (PER42) (P42) (PRXR1)
At2g41680	-0.68	-1.28	-1.52	-2.16	-2.04	-1.54	thioredoxin reductase
At4g24930	-1.70	-1.06	-0.70	-1.60	-2.62	-1.53	thylakoid lumenal 17.9 kDa protein
At2g29310	-1.12	-1.59	-2.61	-2.65	0.30	-1.53	tropinone reductase
At1g14480	-1.00	-0.55	-2.43	-2.16		-1.53	ankyrin repeat family protein
At1g73780	-0.17	-1.10	-1.22	-3.64		-1.53	protease inhibitor/seed storage/LTP
At3g51600	-1.79	-1.33	-1.12	-2.10	-1.33	-1.53	nonspecific lipid transfer protein 5 (LTP5)
At1g76450	-0.87	-0.79	-0.51	-2.69	-2.79	-1.53	oxygen-evolving complex-related
At5g47110	-0.26	-0.21	-1.25	-3.27	-2.65	-1.53	lil3 protein
At5g44770	-2.03	-1.28	-3.23	-1.38	0.28	-1.53	DC1 domain-containing protein
At1g75160	-0.56	-1.75	-2.73	-1.07		-1.53	expressed protein
At5g06530	-1.17	-0.87	-3.07	-1.18	-1.35	-1.53	ABC transporter family protein
At1g20970	-1.81	-1.26	-1.51			-1.53	adhesin-related
At4g36250	-1.84	-1.89	-1.69		-0.67	-1.52	aldehyde dehydrogenase family protein
At2g40670	-0.90	-1.44	-2.97	-0.78		-1.52	two-component responsive regulator
At2g20260	-1.15	-1.96	-1.78	-2.56	-0.15	-1.52	photosystem I reaction center subunit IV
At2g23000	-0.01	-0.34	-2.31		-3.41	-1.52	serine carboxypeptidase S10 family protein
At5g01020	-0.86	-1.57		-2.46	-1.19	-1.52	protein kinase family protein
At3g55800	-0.50	-1.61	-0.09	-2.63	-2.76	-1.52	sedoheptulose-1,7-bisphosphatase
At2g33800	-1.33	-1.23	-0.86	-2.30	-1.88	-1.52	ribosomal protein S5 family protein
At5g48545		-0.38	-0.68	-3.08	-1.92	-1.52	histidine triad family protein / HIT family protein
At5g62690	-0.82	-2.07	-1.73	-2.17	-0.79	-1.51	tubulin beta-2/beta-3 chain (TUB2)
At5g39310	-2.10	-2.21	-2.33	-0.82	-0.12	-1.51	expansin (EXP24)
At1g18730	-0.79	-1.01	-0.29	-2.87	-2.60	-1.51	expressed protein
At1g63960	-1.39	-0.76	-0.34	-2.82	-2.26	-1.51	hypothetical protein
At2g28410	-1.63	-1.70	-2.12	-1.26	-0.86	-1.51	expressed protein
At4g16810	-1.08	-2.43	-1.66	-0.84	-1.55	-1.51	expressed protein
At2g21170	-1.11	-0.57	-1.05	-2.55	-2.27	-1.51	triosephosphate isomerase
At4g30440	-1.10	-2.26	-1.46	-2.19	-0.54	-1.51	UDP-D-glucuronate 4-epimerase
At3g11170	-1.33	-1.54	-0.18	-2.67	-1.83	-1.51	omega-3 fatty acid desaturase
At1g11655	-1.85	-2.08	-2.19	-1.32	-0.10	-1.51	expressed protein
At1g66970	-1.34	-1.20	-1.28	-2.40	-1.31	-1.51	glycerophosphoryl diester phosphodiesterase family
At1g29420	-2.09	-1.64	-0.80			-1.51	auxin-responsive family protein
At3g48100	-1.67	0.62	-1.78		-3.19	-1.51	two-component responsive regulator
At5g18010	-1.62	0.14	-0.45	-2.64	-2.93	-1.50	auxin-responsive protein
At2g28440	-0.20	-1.54	-1.09	-2.20	-2.48	-1.50	proline-rich family protein
At5g47760	-1.52		-3.43	-0.07	-0.98	-1.50	phosphoglycolate phosphatase

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At4g15110	-0.86	-1.44		-2.06	-1.65	-1.50	cytochrome P450 97B3
At2g35260	-1.36	-0.25	-1.78	-2.42	-1.69	-1.50	expressed protein
At4g00400	-0.27	-0.26	-1.18	-3.17	-2.61	-1.50	phospholipid/glycerol acyltransferase
At1g72030	-0.78	-0.76	-0.06	-2.53	-3.34	-1.50	GCN5-related N-acetyltransferase
At4g16155	-0.48	-1.38	-1.81	-2.27	-1.54	-1.50	dihydrolipoamide dehydrogenase 2
At5g65220	-0.78	-1.38	-0.18	-3.09	-2.04	-1.49	ribosomal protein L29 family protein
At5g56980	-0.28	-0.04	-3.08	-2.59	-1.47	-1.49	expressed protein
At2g01870		-1.37	-1.47	-1.85	-1.28	-1.49	expressed protein
At1g24230	-1.33	-1.87	-1.27			-1.49	paired amphipathic helix repeat-containing protein
At1g11790	-0.63		-2.15		-1.69	-1.49	prephenate dehydratase family protein
At3g19320	-1.39	-2.12	-0.96			-1.49	leucine-rich repeat family protein
At3g01360	-1.13	-2.11	-2.17	-0.94	-1.09	-1.49	expressed protein
At4g33660	-0.35	-1.11	-2.03		-2.46	-1.49	expressed protein
At5g22040	-0.99	-1.49	-2.26	-1.58	-1.11	-1.49	expressed protein
At3g52230	-0.53	-1.26		-1.89	-2.27	-1.48	expressed protein
At1g32200	-1.72	-1.30	-1.63	-2.01	-0.76	-1.48	glycerol-3-phosphate acyltransferase
At3g29240	-0.37	-1.46	-2.02	-1.76	-1.8	-1.48	expressed protein
At1g77760	-1.32	0.34	-1.23	-2.37	-2.84	-1.48	nitrate reductase 1 (NR1)
At1g01730	-1.60	-1.27	-0.77	-1.73	-2.02	-1.48	zinc finger (DHHC type) family protein
At2g29320	-0.67	-1.85	-0.30	-3.09	-1.48	-1.48	tropinone reductase
At2g41940	-0.44	-1.25	-1.24	-2.31	-2.16	-1.48	zinc finger (C2H2 type) family protein
At1g30825	-0.43	-0.67	-1.47		-3.34	-1.48	actin-related
At5g22380	-1.91		-0.63		-1.89	-1.48	no apical meristem (NAM) family protein
At3g52380	-1.55	-0.44	-0.18	-2.96	-2.26	-1.48	33 kDa ribonucleoprotein
At4g13930	-0.76	-2.11	-0.08	-2.16	-2.27	-1.48	glycine hydroxymethyltransferase
At3g56040	-1.27	-1.71	-0.73	-2.15	-1.53	-1.48	expressed protein
At2g38480	-0.79	-0.65	-1.82		-2.63	-1.47	integral membrane protein
At5g62700	-0.81	-2.00	-1.51	-2.26	-0.79	-1.47	tubulin beta-2/beta-3 chain (TUB3)
At4g15680	-1.10	0.53			-3.85	-1.47	glutaredoxin family protein
At2g30570	-1.51	-1.43	-0.87	-2.60	-0.89	-1.46	photosystem II reaction center W
At5g33393	-1.24	-2.04	-1.10			-1.46	hypothetical protein
At1g02150	-0.70	0.18	-3.66	-1.92	-1.19	-1.46	pentatricopeptide (PPR) repeat-containing protein
At5g19760	-0.43	-1.19	-1.28	-2.23	-2.16	-1.46	dicarboxylate/tricarboxylate carrier
At1g78070	-0.92	-0.90	-0.49	-2.06	-2.91	-1.46	WD-40 repeat family protein
At2g35500	-2.09	-0.19	-1.20	-3.24	-0.56	-1.46	shikimate kinase-related
At5g09660	-0.01	-2.18	0.27	-2.48	-2.88	-1.45	malate dehydrogenase
At2g36870	-0.40	-1.16	-2.27		-2	-1.45	xyloglucan:xyloglucosyl transferase
At5g42030	-1.35	-1.50	-1.97	-1.89	-0.55	-1.45	expressed protein
At5g08280	-0.83	-1.36	-1.19	-2.09	-1.78	-1.45	hydroxymethylbilane synthase
At2g38540	-1.86	-0.01	-0.20	-3.80	-1.38	-1.45	nonspecific lipid transfer protein 1 (LTP1)
At1g29930	-1.85	-0.71	-1.18	-3.30	-0.19	-1.45	chlorophyll A-B binding protein 2
At1g33640	-1.17	-1.93	-0.53	-1.24	-2.36	-1.45	hypothetical protein
At5g62840	-1.50	0.43			-3.27	-1.45	phosphoglycerate
At3g21465	-0.39	-2.72	-0.13	-2.54		-1.44	expressed protein
At1g79840	-1.58	-2.29	-1.23	-0.94	-1.18	-1.44	homeobox-leucine zipper protein 10 (HB-10)
At4g25080	-1.31		-0.07	-3.16	-2.66	-1.44	magnesium-protoporphyrin O-methyltransferase
At5g38130	-0.52	-0.37	-3.44			-1.44	transferase family protein
At3g20790	-1.12	-1.39	-0.15		-3.11	-1.44	oxidoreductase family protein
At5g07720	-0.98	-1.33	-1.07		-2.39	-1.44	galactosyl transferase GMA12
At4g33840	-1.85	-1.58	-0.89			-1.44	glycosyl hydrolase family 10 protein
At3g07530	-0.96	-0.25	-3.11			-1.44	expressed protein
At1g06950	-0.91	-0.97	-2.41		-1.46	-1.44	chloroplast inner envelope protein-related
At5g26850	-1.00	-1.29	-1.00	-2.13	-1.76	-1.44	expressed protein
At1g69770	-1.73	-1.46	-3.51	-0.58	0.08	-1.44	chromomethylase 3
At3g57120	-0.41	-1.68	-0.87	-2.06	-2.16	-1.44	protein kinase family protein
At1g57790	-0.41	-0.76	-1.85	-3.18	-0.97	-1.43	F-box family protein
At5g06060	-1.34	-1.07	0.32	-3.38	-1.69	-1.43	tropinone reductase
At4g27595	-1.11	-1.39	-1.78			-1.43	protein transport protein-related
At1g76100	-1.10	-1.18	-0.05	-2.84	-1.99	-1.43	plastocyanin
At1g75100	-0.77	-0.78	-1.58	-1.06	-2.96	-1.43	expressed protein
At5g39830	-0.66	-0.54	-2.53	-1.36	-2.05	-1.43	DegP protease
At5g25630	-0.89	-1.02	-2.38	-1.54	-1.32	-1.43	pentatricopeptide (PPR) repeat-containing protein
At4g30990	-1.09	-0.76	0.03		-3.88	-1.42	expressed protein
At5g16230	-0.85	-0.05	-3.38			-1.42	acyl-[acyl-carrier-protein] desaturase
At5g13140	-0.98	-0.46	-0.45	-2.88	-2.34	-1.42	expressed protein
At5g19370	-0.80	-1.32	-1.43	-2.05	-1.51	-1.42	rhodanese-like domain-containing protein
At5g46020	-0.51	-2.41	-2.74	-0.65	-0.81	-1.42	expressed protein
At5g23970	-1.17	-0.92	-0.65	-3.05	-1.31	-1.42	transferase family protein
At3g57050	-1.36	-1.22		-2.11	-0.98	-1.42	cystathionine beta-lyase
At1g72440	-1.25	-1.54	-0.78	-2.41	-1.10	-1.42	CCAAT-box-binding transcription factor-related
At3g11630	-0.20	-0.21	-1.03	-3.21	-2.43	-1.42	2-cys peroxiredoxin
At2g37260	-0.27		-1.82		-2.16	-1.42	WRKY family transcription factor
At1g55910	-0.99	-0.60	-3.08	-1.41	-0.99	-1.41	metal transporter

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At4g08480	-1.11	-1.41	-2.72	-1.38	-0.45	-1.41	mitogen-activated protein kinase
At1g54500	-1.72	-1.10	0.51	-2.55	-2.20	-1.41	rubredoxin family protein
At1g62120	-0.04	0.35	-2.27	-2.55	-2.55	-1.41	mitochondrial transcription termination factor-related
At3g59400	-0.83	-0.70	-1.32	-2.09	-2.11	-1.41	expressed protein
At4g37100	-1.33	-0.96			-1.94	-1.41	hypothetical protein
At5g55210	-1.46		-1.71	-1.75	-0.72	-1.41	expressed protein
At5g36280	-0.51	-0.63	-0.47	-4.04		-1.41	hypothetical protein
At3g18490	-1.25	-1.19	-1.35	-1.62	-1.64	-1.41	aspartyl protease family protein
At1g12460	-0.35	-1.55	-2.50	-1.24		-1.41	leucine-rich repeat transmembrane protein kinase
At2g03710	-0.42	-1.20	-1.85	-0.84	-2.72	-1.41	MADS-box protein (AGL3)
At1g70895	-1.37	-0.49	-3.50		-0.27	-1.41	CLE17
At2g24395	-0.55	-1.01	-0.38	-2.42	-2.66	-1.41	chaperone protein dnaJ-related
At5g04680	-1.62	-1.90	-0.84	-1.67	-1.01	-1.41	expressed protein
At4g26600	-0.81	-1.64		-0.19	-2.98	-1.40	nucleolar protein
At4g39970	-0.81	-0.56	-0.69	-2.47	-2.49	-1.40	haloacid dehalogenase-like hydrolase family protein
At2g40250	-1.12	-1.67	-1.42			-1.40	GDSL-motif lipase/hydrolase family protein
At4g28730	-0.87	-0.17	-0.91	-1.68	-3.39	-1.40	glutaredoxin family protein
At3g20820	-1.43	-1.94	-1.14	-1.92	-0.58	-1.40	leucine-rich repeat family protein
At3g14670	-1.94	-2.16	-0.11			-1.40	hypothetical protein
At2g28250	-1.93	-0.28	-0.56		-2.83	-1.40	protein kinase family protein
At1g68660	-0.92	-0.04	-1.71	-1.85	-2.49	-1.40	expressed protein
At5g06590	-0.57	-2.12	-1.51			-1.40	expressed protein
At5g67130	-0.51	-0.31	-2.54	-2.25	-1.39	-1.40	expressed protein
At3g61100	-1.37	-1.49	-0.88	-2.51	-0.74	-1.40	expressed protein
At1g36510	-1.43	-1.25	-1.51			-1.40	hypothetical protein
At3g28910	-1.10	-0.86	-1.31		-2.31	-1.40	myb family transcription factor (MYB30)
At1g05085	-1.26	-2.77	-2.42		0.87	-1.40	hypothetical protein
At1g50730	-1.26	-0.93	-0.53	-1.77	-2.49	-1.40	expressed protein
At2g43550	-0.84	-0.70	-1.82	-3.15	-0.46	-1.39	trypsin inhibitor
At1g74850	-1.59	-1.89	-1.11	-1.15	-1.22	-1.39	pentatricopeptide (PPR) repeat-containing protein
At1g65360	-0.98	-0.05	-3.15			-1.39	MADS-box protein (AGL23)
At3g27180	-1.18	-1.51	-1.28	-1.92	-1.06	-1.39	expressed protein
At1g01980	-0.92	-0.60	-2.65			-1.39	FAD-binding domain-containing protein
At1g52590	-1.23	-0.76	-1.37	-1.62	-1.94	-1.38	expressed protein
At4g10300	-1.30	-0.78	-1.09	-1.85	-1.90	-1.38	expressed protein
At3g57180	-1.33	-0.46			-2.36	-1.38	expressed protein
At2g44520	-1.57	-1.79	-3.11		-0.45	-1.38	UbiA prenyltransferase family protein
At1g64770	-1.30	-1.34	-0.22	-1.96	-2.09	-1.38	expressed protein
At1g31860	-0.75	-0.90	-3.69	-0.86	-0.72	-1.38	histidine biosynthesis bifunctional protein
At4g01900	-0.40	-0.46	-3.69	-1.67	-0.69	-1.38	P II nitrogen sensing protein (GLB I)
At2g44920	-0.93	-0.84	-1.43	-2.28	-1.43	-1.38	thylakoid lumenal 15 kDa protein
At1g04030	-0.46	0.22	-2.51		-2.78	-1.38	expressed protein
At1g09310	-0.73	-1.06	-0.12	-2.78	-2.21	-1.38	expressed protein
At4g30620	-1.43	-0.95	-0.16	-2.19	-2.17	-1.38	expressed protein
At3g29660	-1.29	-0.37	-1.11		-2.75	-1.38	hypothetical protein
At3g26710	-0.84	-1.82	-0.42	-1.86	-1.94	-1.38	expressed protein
At3g06145	-1.56	-0.66	-0.38	-2.35	-1.93	-1.38	expressed protein
At3g45780	-0.79	-1.19	-3.43	-1.66	0.19	-1.37	protein kinase
At4g08640	-1.21	-1.10	-1.81			-1.37	hypothetical protein
At5g13670	-0.78	-0.69	-2.65			-1.37	nodulin MtN21 family protein
At1g63900	-1.12	-2.05	-2.43	-0.74	-0.53	-1.37	zinc finger (C3HC4-type RING finger) family protein
At3g49550	-1.79	-1.68	-2.30	-0.83	-0.27	-1.37	expressed protein
At1g18310	-0.58	-0.28	-3.26			-1.37	glycosyl hydrolase family 81 protein
At1g62780	-0.74	-1.46	-0.31	-1.91	-2.43	-1.37	expressed protein
At5g36150	-0.88	-1.86	-0.87	-1.48	-1.77	-1.37	pentacyclic triterpene synthase
At5g39530	-0.80	-1.21	-0.74	-2.73		-1.37	expressed protein
At4g05340	-1.35	0.62	-3.30	-1.68	-1.12	-1.37	hypothetical protein
At2g04700	-1.08	-1.07	-0.83	-2.13	-1.73	-1.37	ferredoxin thioredoxin reductase
At3g53460	-1.15	-1.07	-0.18	-2.47	-1.96	-1.37	29 kDa ribonucleoprotein
At2g28800	-0.81	-1.07	-1.33	-1.68	-1.93	-1.37	chloroplast membrane protein
At4g13600	-0.25	-0.96	-2.89			-1.37	glycosyl hydrolase family protein 17
At2g22125	-1.04	-1.29	-1.55	-2.03	-0.91	-1.37	C2 domain-containing protein
At3g07270	-0.06	-0.41	-3.60	-1.30	-1.45	-1.36	GTP cyclohydrolase I
At1g29500	-1.19	-0.72	-0.75	-2.79		-1.36	auxin-responsive protein
At5g66530	-0.85	-0.36	-0.45	-3.16	-1.99	-1.36	aldose 1-epimerase family protein
At1g31330	-1.12	-1.99	-0.83	-2.29	-0.58	-1.36	photosystem I reaction center subunit III
At2g35840	-0.55	-1.35	-1.43	-1.30	-2.18	-1.36	sucrose-phosphatase 1 (SPP1)
At3g54210	-0.30	-0.98	-0.65	-2.42	-2.46	-1.36	ribosomal protein L17 family protein
At2g24740	-0.75	-1.13	-0.03	-2.54	-2.35	-1.36	SET domain-containing protein (SUVH8)
At2g02790	-0.62	-0.64	-2.81			-1.36	calmodulin-binding family protein
At5g51020		0.24	-1.90	-1.76	-2.02	-1.36	expressed protein
At3g48420	-1.50	-0.30	-0.22	-2.49	-2.29	-1.36	haloacid dehalogenase-like hydrolase family protein
At1g06690	-1.03	-1.08		-1.43	-1.89	-1.36	aldo/keto reductase family protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At4g30110	-0.71	-1.30	-1.20	-0.22	-3.36	-1.36	ATPase E1-E2 type family protein
At5g06240	-1.01	-1.78	-0.56	-1.67	-1.77	-1.36	expressed protein
At1g67480	-0.69	-2.34	-0.36	-2.17	-1.23	-1.36	kelch repeat-containing F-box family protein
At5g53500			-2.45	-1.12	-0.50	-1.36	WD-40 repeat family protein
At3g54250	-1.21	-1.89	-0.97			-1.36	mevalonate diphosphate decarboxylase
At5g24190	-1.53	-2.13	-0.41			-1.36	hypothetical protein
At3g26900	-1.06	-1.21	0.39	-2.78	-2.10	-1.35	shikimate kinase family protein
At1g32520	-0.48	-0.76	-0.25	-2.14	-3.15	-1.35	expressed protein
At1g12560	-1.21	-1.72	-1.40		-1.08	-1.35	expansin (EXP7)
At1g17650	-0.43	-0.80	-0.81	-2.81	-1.92	-1.35	6-phosphogluconate dehydrogenase
At3g03300	-0.85	-0.80	-3.71	-0.73	-0.68	-1.35	DEAD/DEAH box helicase
At3g61040	0.06	-0.53	-2.90		-2.04	-1.35	cytochrome P450 family protein
At2g05790	-0.36	-1.59	-1.20		-2.25	-1.35	glycosyl hydrolase family 17 protein
At1g45474	-0.97	-0.83	0.04	-1.91	-3.08	-1.35	chlorophyll A-B binding protein
At2g28190	-0.33	-0.79	-1.25	-1.81	-2.57	-1.35	superoxide dismutase
At5g63180	-1.82	-1.17	-0.34	-1.46	-1.95	-1.35	pectate lyase family protein
At5g49730	0.31	-0.53	-1.10	-3.15	-2.27	-1.35	ferric reductase-like
At3g54600	-1.15	-1.45	0.06	-2.24	-1.96	-1.35	DJ-1 family protein
At4g17600	-0.55	-0.80	-1.42	-1.99	-1.98	-1.35	lil3 protein
At4g23740	-1.85	-1.43	-0.32		-1.79	-1.35	leucine-rich repeat transmembrane protein kinase
At3g22120	-1.19	-1.95	-2.60	-1.46	0.45	-1.35	protease inhibitor/seed storage/LTP
At3g03773	-1.07	-0.94	-2.07	-1.09	-1.57	-1.35	expressed protein
At4g22410	-0.99	-2.01	-0.18	-1.78	-1.78	-1.35	ubiquitin carboxyl-terminal hydrolase family protein
At5g66052	-1.02	-0.48	-1.65	-1.95	-1.62	-1.35	expressed protein
At3g25530	-1.13	-1.16	-0.26	-2.89	-1.28	-1.34	6-phosphogluconate dehydrogenase
At5g40850	-1.02	-0.58	-0.50	-2.57	-2.03	-1.34	urophorphyrin III methylase (UPM1)
At4g22010		-1.22	-0.46		-2.34	-1.34	multi-copper oxidase type I family protein
At3g58610	-0.86	-1.20	-0.33	-2.28	-2.03	-1.34	ketol-acid reductoisomerase
At1g47580	-1.40	-0.56	-0.70	-1.96	-2.07	-1.34	lipoyltransferase
At5g08650	-1.39	-0.32	-0.89	-2.32	-1.77	-1.34	GTP-binding protein LepA
At2g41470	-0.48	-1.41	-1.40	-1.96	-1.43	-1.34	embryo-specific protein-related
At5g15350	-1.56	-0.77	-0.38	-1.53	-2.43	-1.33	plastocyanin-like domain-containing protein
At1g29920	-1.53	-1.10			-1.38	-1.33	chlorophyll A-B binding protein
At2g37450	-0.99	-1.07	-1.94			-1.33	nodulin MtN21 family protein
At2g32870	-0.67	-1.02	-2.49	-2.58	0.10	-1.33	meprin and TRAF homology domain-containing protein
At5g48670	-2.03	-1.13	0.70	-2.86		-1.33	MADS-box family protein
At1g31420	-1.11	-1.41	-1.33	-1.77	-1.02	-1.33	leucine-rich repeat transmembrane protein kinase
At2g40570	0.01	-1.03	-2.95			-1.33	initiator tRNA phosphoribosyl transferase family protein
At5g25950	-1.51	-0.94	-1.53			-1.33	hypothetical protein
At2g23620	-1.73	-1.78	-0.74		-1.05	-1.32	esterase
At1g68400	-1.03	-0.42	-0.49		-3.36	-1.32	leucine-rich repeat transmembrane protein kinase
At5g27070	-1.58	-0.27	-2.13			-1.32	MADS-box family protein
At3g12890	-0.61	-1.15	-2.21			-1.32	expressed protein
At5g61650	-0.86	-0.62	-2.86	-0.94		-1.32	cyclin family protein
At2g35860	-0.95	-1.21	-1.43		-1.69	-1.32	beta-Ig-H3 domain-containing protein
At5g03880	-1.13	-1.25	-0.15	-2.54	-1.52	-1.32	expressed protein
At5g25460	-0.65	-1.44	-2.48		-0.70	-1.32	expressed protein
At1g48300	-0.77	-1.09	-1.83	-1.36	-1.54	-1.32	expressed protein
At1g80140	-1.60	-2.52	-1.35	-0.70	-0.40	-1.31	glycoside hydrolase family 28 protein
At1g04550	-0.80	-0.51	-2.14		-1.80	-1.31	auxin-responsive protein
At5g08330	-0.92	-1.56	-1.91		-0.86	-1.31	TCP family transcription factor
At5g13400	-2.47	-0.54	0.15		-2.39	-1.31	POT family protein
At5g14210		-1.07	-0.29	-2.24	-1.66	-1.31	leucine-rich repeat transmembrane protein kinase
At5g57840	-1.35	-1.30	-2.19	-0.90	-0.82	-1.31	transferase family protein
At5g14100	-1.06	-0.66	0.44	-3.04	-2.24	-1.31	ABC transporter family protein
At5g66470	-1.37	-1.13	-0.58	-2.04	-1.43	-1.31	expressed protein
At1g65500	-0.87	-1.58	-3.20	0.03	-0.93	-1.31	expressed protein
At3g50530	-0.71	-2.95	-1.51	-0.47	-0.91	-1.31	calcium-dependent protein kinase
At2g41010	-0.69	-1.01	-0.68	-2.86		-1.31	VQ motif-containing protein
At5g60020	-0.49	-1.81	-1.63			-1.31	laccase
At4g32570	-1.17	-0.33	-0.33	-2.56	-2.16	-1.31	expressed protein
At1g02205	-1.30	-0.61	-0.41	-2.11	-2.10	-1.31	CER1 protein
At5g10690	-1.88	-0.36	-0.46		-2.52	-1.31	pentatricopeptide (PPR) repeat-containing protein
At3g61035	-1.47	-1.84	-1.64	-1.85	0.26	-1.31	cytochrome P450 family protein
At4g38660	-1.07	-0.70	-0.76		-2.69	-1.31	thaumatin
At2g42260	-1.41	-1.06	-1.45			-1.30	expressed protein
At1g34470	-0.57	-1.72	-1.44	-1.67	-1.11	-1.30	permease-related
At3g49140	-1.13	-0.86	-1.00	-1.92	-1.62	-1.30	pentatricopeptide (PPR) repeat-containing protein
At5g03090	-1.26	-2.15	-2.08	-0.94	-0.08	-1.30	hypothetical protein
At1g47230	-0.49	-0.62	-1.10	-3.34	-0.95	-1.30	cyclin
At1g06200	-0.64	-1.13	-0.71	-2.41	-1.61	-1.30	expressed protein
At2g33255	-0.85	-1.32	-0.87		-2.17	-1.30	haloacid dehalogenase-like hydrolase family protein
At1g27030	-1.60	-1.44	-1.85		-0.31	-1.30	expressed protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At1g70070	-0.64	-1.20	-0.85	-0.64	-3.18	-1.30	DEAD/DEAH box helicase
At4g31290	-1.31	-1.00	-2.30	-2.18	0.31	-1.30	ChaC-like family protein
At5g54290	-1.31	-0.83	-0.63	-2.43	-1.28	-1.30	cytochrome c biogenesis protein
At3g63490	-0.59	-0.42	-2.00	-2.11	-1.36	-1.30	ribosomal protein L1 family protein
At5g54240	-1.46	-1.36	-1.32	-0.76	-1.59	-1.30	expressed protein
At2g02120	-1.01	-1.19	-2.80		-0.19	-1.30	plant defensin-fusion protein
At2g39090		-1.60	-1.93		-0.36	-1.30	tetratricopeptide repeat
At1g23740	-0.84	-0.27	-0.31	-1.90	-3.15	-1.29	oxidoreductase,n
At4g01080	-0.86	-0.96	-0.46	-1.78	-2.42	-1.29	expressed protein
At1g52870	-1.19	-0.62	-1.51	-1.46	-1.69	-1.29	peroxisomal membrane protein-related
At5g59480	-0.38	-1.20	-0.41	-1.73	-2.74	-1.29	haloacid dehalogenase-like hydrolase family protein
At5g34887	-0.77	-1.63	-1.48			-1.29	expressed protein
At2g14820	-0.07	-1.91	-0.82		-2.37	-1.29	phototropic-responsive NPH3 family protein
At1g68900	-1.11	-0.59	0.05	-2.53	-2.27	-1.29	mandelate racemase
At2g27290	-0.44	-1.38	-1.59	-1.60	-1.43	-1.29	expressed protein
At5g47540	-1.78	-0.04	-1.56		-1.78	-1.29	Mo25 family protein similar to MO25 protein
At1g51110	-1.25	0.08	-0.31	-3.00	-1.97	-1.29	plastid-lipid associated protein PAP
At3g19030	-0.17	-0.32	-1.83	-2.33	-1.81	-1.29	expressed protein
At4g26250	-1.38	-2.70			0.21	-1.29	galactinol synthase
At4g38840	-1.50	-0.35	0.02	-2.55	-2.05	-1.29	auxin-responsive protein
At2g41560	-1.89	-1.30	-0.11	-1.81	-1.33	-1.29	calcium-transporting ATPase 4
At4g27600	-0.68	-1.99	-0.40	-1.62	-1.74	-1.28	pfkB-type carbohydrate kinase family protein
At1g51730	-0.51	-0.78	-1.25	-1.69	-2.2	-1.28	RWD domain-containing protein
At1g74880	-1.21	-0.89	-0.76	-1.71	-1.85	-1.28	expressed protein
At2g45400	-1.06	-2.44	-0.79	-0.11	-2.02	-1.28	dihydroflavonol 4-reductase family protein
At1g48750	-0.42	-0.46	-2.16	-1.43	-1.95	-1.28	protease inhibitor/seed storage/LTP
At1g20470	-0.39	-1.70	-0.99	-1.08	-2.25	-1.28	auxin-responsive family protein
At1g18170	-0.52	-1.38	-0.90	-2.39	-1.21	-1.28	immunophilin
At5g62350	-1.54	-1.47	-0.52	-2.89	0.01	-1.28	invertase/pectin methylesterase inhibitor family protein
At1g66940	-1.41	-0.49	-1.79	-2.06	-0.65	-1.28	protein kinase-related
At1g75090	-1.60	-1.59	-1.26		-0.67	-1.28	methyladenine glycosylase family protein
At2g32350	-1.49	-0.79	-0.88	-1.95	-1.28	-1.28	ubiquitin family protein
At2g01620	-0.44	-0.64	-2.57	-1.52	-1.21	-1.28	expressed protein
At5g10170	-0.90	-0.60	-0.51	-2.62	-1.76	-1.28	inositol-3-phosphate synthase
At3g48960	-1.97	-1.14	-0.73			-1.28	60S ribosomal protein L13
At2g22170	-0.93	-1.06	0.06	-2.99	-1.47	-1.28	lipid-associated family protein
At2g19170	-1.13	-1.24	-0.71	-2.19	-1.10	-1.27	subtilase family protein
At4g36070	-0.62	-1.20	-2.00			-1.27	calcium-dependent protein kinase
At4g04340	-1.34	-1.02		-2.52	-0.22	-1.27	early-responsive to dehydration protein-related
At4g34610	-1.53	-0.54		-1.36	-1.66	-1.27	homeodomain-containing protein
At1g09665	-2.43	-2.14	-0.38	-0.97	-0.43	-1.27	Toll-Interleukin-Resistance
At1g67150	-1.01	-1.5	0.13	-1.97	-1.99	-1.27	hypothetical protein
At5g01920	-0.79	-0.65	-1.22	-2.05	-1.62	-1.27	protein kinase family protein
At1g77400	-0.44	-1.49	-1.69	-1.39	-1.33	-1.27	expressed protein
At4g28270	-0.84	-0.90	-2.35	-2.04	-0.19	-1.27	zinc finger (C3HC4-type RING finger) family protein
At3g18773		-0.73	-0.38	-1.96	-1.99	-1.26	zinc finger (C3HC4-type RING finger) family protein
At3g24720	0.21	-1.95	-1.39	-1.93		-1.26	protein kinase family protein
At3g15360	-0.09	0.07	-1.74	-2.96	-1.58	-1.26	thioredoxin M-type 4
At4g07825	-0.84	-0.94		-1.92	-1.36	-1.26	expressed protein
At5g14760	-0.72	-0.25		-1.46	-2.62	-1.26	L-aspartate oxidase family protein
At4g38280	-0.76	-0.83	-2.20			-1.26	expressed protein
At2g29130	-1.54	-0.49	-1.75			-1.26	laccase
At3g23940	-1.63	-0.56	0.36	-2.87	-1.60	-1.26	dehydratase family protein
At2g43750	-1.27	-0.49	-0.12	-2.57	-1.85	-1.26	cysteine synthase
At3g17370	-2.66	-1.33	0.21			-1.26	pentatricopeptide (PPR) repeat-containing protein
At2g27230	-1.33	-1.25	-1.92	-0.80	-0.99	-1.26	transcription factor-related
At1g09870	-1.12	-1.17		-1.93	-0.82	-1.26	histidine acid phosphatase family protein
At3g54660	-0.34	-0.87	-1.73	-1.03	-2.33	-1.26	gluthatione reductase
At4g18590	-0.88	-0.61	-1.14	-2.47	-1.19	-1.26	expressed protein
At2g23910	-1.22	-1.81	-0.66		-1.34	-1.26	cinnamoyl-CoA reductase-related
At3g53750	-0.70	-0.44	-0.84	-2.00	-2.31	-1.26	actin 3 (ACT3)
At2g30950	-0.50	-1.02	-1.20	-1.75	-1.82	-1.26	FtsH protease (VAR2)
At5g06700	-0.74	-1.66	-2.44	-1.28	-0.15	-1.25	expressed protein
At4g31310	-0.75	-0.07	-0.53	-2.76	-2.15	-1.25	avirulence-responsive protein-related
At5g46560	-0.79	-1.57	-1.17	-1.48	-1.24	-1.25	expressed protein
At1g55160	-0.37	-0.85	-0.52	-2.77	-1.76	-1.25	expressed protein
At5g13410	-0.92	-0.62	-1.23	-2.34	-1.13	-1.25	immunophilin
At4g18780	-1.00	-1.17	-2.13		-0.7	-1.25	cellulose synthase
At1g16650		-0.28		-1.44	-2.03	-1.25	expressed protein
At5g24165	-0.46	-0.87	-0.82	-1.73	-2.34	-1.25	expressed protein
At1g13280	-0.54	-1.03	-0.20	-2.87	-1.60	-1.25	allene oxide cyclase family protein
At1g11960	-0.75	-1.39	-0.83		-2.02	-1.25	early-responsive to dehydration protein-related
At4g05330	-0.72	-0.11	-3.23		-0.92	-1.25	zinc finger and C2 domain protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At5g08620	-0.68	0.04			-3.10	-1.25	DEAD box RNA helicase
At5g28930	-0.63	-0.14	-2.97			-1.25	hypothetical protein
At1g19050	-1.20	-0.42	-1.01	-1.14	-2.46	-1.24	two-component responsive regulator
At5g41200	-1.82	-0.66	-2.53		0.03	-1.24	MADS-box family protein
At5g46420	-0.59	-0.59	-0.74	-2.68	-1.63	-1.24	16S rRNA processing protein
At2g19940	-0.95	-0.23	-0.32	-3.06	-1.66	-1.24	semialdehyde dehydrogenase family protein
At4g14060	0.08	-0.49	-1.18	-1.98	-2.65	-1.24	major latex protein-related
At2g31790	-1.59	-1.24	-0.90			-1.24	UDP-glucosyl transferase family protein
At5g18140	-0.96	-0.50	-0.59	-2.87	-1.29	-1.24	DNAJ heat shock N-terminal
At3g03630	-1.58	0.08	-1.00		-2.46	-1.24	cysteine synthase
At3g06650	-0.72	-0.85	-0.32	-1.36	-2.96	-1.24	ATP-citrate synthase
At2g22730	-0.79	-0.27	-3.23	-1.32	-0.58	-1.24	transporter-related
At1g14970		-1.47	-0.38	-1.99	-1.11	-1.24	expressed protein
At2g25480	-0.93	-1.18	0.51	-2.10	-2.48	-1.24	expressed protein
At5g23790	-1.17	-0.93	-0.82	-2.16	-1.10	-1.24	galactinol synthase
At1g01120	-0.95	-1.81	-1.00	-1.58	-0.85	-1.24	fatty acid elongase 3-ketoacyl-CoA synthase 1
At3g55250	-0.40	-1.33	-0.40	-2.78	-1.27	-1.24	expressed protein
At4g15093	0.45	-1.96	-1.22	-2.21		-1.23	catalytic LigB subunit
At4g21445	-0.76	-0.94	-1.36		-1.88	-1.23	expressed protein
At1g79660	-0.50	-0.25	-2.93	-1.81	-0.68	-1.23	expressed protein
At2g05562	-0.63	-0.27	-2.87	-0.39	-2.01	-1.23	hypothetical protein
At3g52840	-1.10	-0.97	-1.36	-1.49	-1.25	-1.23	beta-galactosidase
At1g68780	-1.02	-1.08	-1.60			-1.23	leucine-rich repeat family protein
At4g27540	0.17	0.07	-2.61	-2.55		-1.23	prenylated rab acceptor (PRA1) protein-related
At3g18010	-0.27	-0.71	-0.80	-1.90	-2.48	-1.23	homeobox-leucine zipper transcription factor
At2g25710	-0.99	-0.69	-1.20	-2.07	-1.20	-1.23	holocarboxylase synthetase 1 (HCS1)
At1g48405	-1.44	-2.08	-0.17			-1.23	hypothetical protein
At5g56050	-1.42	-1.16	-2.47	-1.16	0.05	-1.23	hypothetical protein
At4g15780	-0.92	-0.95	-1.82	-1.83	-0.63	-1.23	synaptobrevin-related family protein
At4g23720	-0.38	-0.44	-2.87			-1.23	expressed protein
At3g48540	-1.00	-1.20	-1.49			-1.23	cytidine/deoxycytidylate deaminase family protein
At2g47910	-0.49	-0.47	-0.78	-1.65	-2.76	-1.23	expressed protein
At2g44160	-0.43	-2.18	-0.31	-1.50	-1.72	-1.23	methylenetetrahydrofolate reductase 2
At3g62320	-2.08	-1.06	-0.53			-1.23	hypothetical protein
At2g26250			-0.29	-1.32	-2.07	-1.23	beta-ketoacyl-CoA synthase
At1g19835	-1.59	-1.64	-1.12		-0.56	-1.23	expressed protein
At1g16080	-1.61	-0.75	0.03	-1.80	-2.00	-1.23	expressed protein
At5g24240	-0.41	-0.20		-2.83	-1.46	-1.22	phosphatidylinositol 3- and 4-kinase family protein
At5g62070	-1.04	-1.35	-2.35	-0.89	-0.50	-1.22	calmodulin-binding family protein
At5g26880	-0.66	-0.22	-2.84	-1.34	-1.06	-1.22	tRNA/rRNA methyltransferase
At2g13360	-0.08	-1.03	-2.05	-1.33	-1.63	-1.22	serine-glyoxylate aminotransferase-related
At1g07110	-0.44	-0.98	-1.32	-1.56	-1.81	-1.22	fructose-6-phosphate 2-kinase
At3g10430	-1.21	-1.01	-1.44			-1.22	F-box family protein
At4g05303	-0.68	-0.69	-2.29			-1.22	hypothetical protein
At2g02500	-1.43	-1.27	-0.96			-1.22	expressed protein
At5g66310	-1.14	-1.08	-1.75	-1.75	-0.38	-1.22	kinesin motor family protein
At4g37660	-0.68	-0.48		-2.58	-1.14	-1.22	ribosomal protein L12 family protein
At4g13170	-1.08	-0.75	-0.06	-2.69	-1.53	-1.22	60S ribosomal protein L13A
At4g33230		-0.31	-1.44	-1.90		-1.22	pectinesterase family protein
At5g10380	-1.20	-0.95		-3.07	-0.87	-1.22	zinc finger (C3HC4-type RING finger) family protein
At3g42722	-0.65	-2.36	-0.64			-1.22	F-box family protein
At5g35790	-0.62	-0.58	-1.52	-1.90	-1.47	-1.22	glucose-6-phosphate 1-dehydrogenase
At1g01620	-0.51	-0.08	-2.59	-1.55	-1.36	-1.22	plasma membrane intrinsic protein 1C
At4g25990	-1.30	-1.04	-1.10	-1.15	-1.50	-1.21	expressed protein
At2g47880	-1.13	-1.02	-1.5			-1.21	glutaredoxin family protein
At3g56830	-0.89	-0.72	-0.97		-2.28	-1.21	expressed protein
At3g04760	-0.88	-1.41	-1.17	-1.02	-1.59	-1.21	pentatricopeptide (PPR) repeat-containing protein
At5g05150	-0.71	-0.48	-2.44			-1.21	transport protein-related
At3g29185	-0.78	-0.56	-1.91	-1.72	-1.08	-1.21	expressed protein
At1g49630	-1.01	-0.29	-1.23	-2.25	-1.27	-1.21	peptidase M16 family protein
At3g57320	-1.20	-1.10	-1.10		-1.44	-1.21	expressed protein
At3g23070	-2.49	-1.07	-1.31	-0.27	-0.91	-1.21	expressed protein
At4g35440	-0.44	-0.81	-0.94		-2.66	-1.21	voltage-gated chloride channel family protein
At5g47810	-0.19	-2.45	-0.99			-1.21	phosphofructokinase family protein
At3g03210	-1.07	-0.89	-1.59	-1.72	-0.77	-1.21	expressed protein
At4g22100	-1.60	-0.15	-0.22	-2.86		-1.21	glycosyl hydrolase family 1 protein
At1g11840	-0.78	-1.35	-0.84	-2.02	-1.04	-1.21	lactoylglutathione lyase
At1g16720	-1.43	-1.16	-0.26	-1.95	-1.23	-1.20	expressed protein
At2g42690	-1.34	-0.65	-0.24	-1.73	-2.05	-1.20	lipase
At2g45310	-0.95	-1.50	-1.66		-0.70	-1.20	UDP-D-glucuronate 4-epimerase
At1g61630	-0.66	-0.79	-1.18	-2.18		-1.20	equilibrative nucleoside transporter
At1g31812	-0.09	-1.76	-2.26	-1.59	-0.32	-1.20	acyl-CoA binding protein
At5g04810	-0.81	-0.59	-1.08	-1.81	-1.72	-1.20	pentatricopeptide (PPR) repeat-containing protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At2g28085	-1.50	-1.57	0.09	-1.87	-1.15	-1.20	auxin-responsive family protein
At1g17050	-1.00	-0.17	-0.24	-1.38	-3.22	-1.20	geranyl diphosphate synthase
At3g08030	-1.24	-0.68	-1.13	-2.93	-0.02	-1.20	expressed protein
At2g06610	-1.13	-1.28	-0.84	-1.62	-1.14	-1.20	hypothetical protein
At3g47580	-0.16	-0.08	-1.08	-3.49		-1.20	leucine-rich repeat transmembrane protein kinase
At2g46930	-0.72	-1.13			-1.74	-1.20	pectinacetylesterase
At4g25740	-0.62	-1.21		-1.84	-1.11	-1.20	40S ribosomal protein S10
At2g06500	-1.68	-0.33	-1.27		-1.50	-1.20	hAT dimerisation domain-containing protein
At5g50030	-1.24	-2.01	-0.33			-1.20	invertase/pectin methylesterase inhibitor family protein
At3g42500	-0.96	-0.05	-0.14	-2.87	-1.95	-1.19	hypothetical protein
At4g27640	-0.96	-0.74	-0.36	-1.91	-2.00	-1.19	importin beta-2 subunit family protein
At4g38225	-0.24	-0.45	-0.92	-2.80	-1.55	-1.19	expressed protein
At1g04390	-1.20	-0.88	-1.50			-1.19	expressed protein
At4g14400	-2.27	-1.70	0.09	-1.58	-0.50	-1.19	ankyrin repeat family protein
At4g22830	-0.07	-0.37	-0.80	-2.66	-2.05	-1.19	expressed protein
At1g80380	-0.81	-1.08	-0.30	-1.92	-1.84	-1.19	phosphoribulokinase/uridine kinase-related
At1g04610	-1.10	-2.21	-1.04	-0.61	-0.98	-1.19	flavin-containing monooxygenase
At1g61180	-1.36	-1.64			-0.57	-1.19	disease resistance protein
At1g07460	-0.54	-0.84	-2.19			-1.19	legume lectin family protein
At3g14940	-0.94	-1.14	-1.16	-0.77	-1.93	-1.19	phosphoenolpyruvate carboxylase
At2g24420	-0.60	-0.84	-2.69	-0.53	-1.28	-1.19	DNA repair ATPase-related
At4g00910	-0.39	-0.69	-0.22	-2.91	-1.72	-1.19	expressed protein
At4g09970	-0.94	-0.65	-1.54	-1.64	-1.16	-1.19	expressed protein
At2g42190	-0.96	-0.90	-1.37	-1.59	-1.10	-1.19	expressed protein
At1g18360	-0.49		-0.33		-2.74	-1.19	hydrolase, alpha/beta fold family protein
At5g22110	0.02		-1.74		-1.83	-1.19	DNA polymerase epsilon subunit B family protein
At3g62530	-0.47	-0.37	-2.60	-1.39	-1.10	-1.18	PBS lyase HEAT-like repeat-containing protein
At2g28180	-1.16	-1.40	-1.00			-1.18	cation/hydrogen exchanger
At1g06510	-0.30	-1.10	-1.25	-2.18	-1.09	-1.18	expressed protein
At4g18240	-1.09	-1.50	-1.62	-0.97	-0.73	-1.18	starch synthase-related protein
At1g78290	-1.42	-0.23	-0.32	-2.05	-1.87	-1.18	serine/threonine protein kinase
At3g01370	-0.55	-1.83			-1.17	-1.18	expressed protein
At5g64140	-0.27	-1.93	-1.95	-1.13	-0.62	-1.18	40S ribosomal protein S28
At2g33330			-0.37	-1.59	-1.58	-1.18	33 kDa secretory protein-related
At2g30310	-0.19	-1.41	0.03	-3.15		-1.18	GDSL-motif lipase/hydrolase family protein
At2g03840	-1.04	-1.37	-0.61	-1.45	-1.42	-1.18	senescence-associated family protein
At5g58330	-0.50	-0.21	-1.04	-1.88	-2.26	-1.18	malate dehydrogenase [NADP]
At1g63220	-1.34	-2.18	-2.29	-0.29	0.22	-1.18	C2 domain-containing protein
At5g14460	-1.17	-2.01	-0.77	-1.09	-0.83	-1.18	pseudouridylate synthase TruB family protein
At5g46290	-1.29	-1.26	-0.25	-1.65	-1.43	-1.17	3-oxoacyl-[acyl-carrier-protein] synthase I
At1g54630	-0.56	-0.35	-3.07	-0.98	-0.91	-1.17	acyl carrier protein 3
At2g20890	-1.01	-0.49	-0.45	-2.14	-1.79	-1.17	expressed protein
At1g50250	-0.38	-0.96	-1.96	-1.03	-1.54	-1.17	cell division protein ftsH homolog 1
At2g25510	-1.22	-2.60	-1.19	-0.77	-0.10	-1.17	expressed protein
At3g53950	-0.62	-0.42	-1.76	-2.12	-0.95	-1.17	glyoxal oxidase-related
At1g17610	-0.83	-1.49	-0.10	-2.27		-1.17	disease resistance protein-related
At4g26790	-1.32	-1.25	-1.14	-1.41	-0.75	-1.17	GDSL-motif lipase/hydrolase family protein
At5g09460	-1.18	-1.63	-0.39	-1.48		-1.17	expressed protein
At4g02530	-0.97	-1.31	0.32	-1.95	-1.95	-1.17	chloroplast thylakoid lumen protein
At1g66130	-0.58	-0.30	-0.54		-3.26	-1.17	oxidoreductase
At3g48610	-0.75	-1.28	-0.36	-1.58	-1.87	-1.17	phosphoesterase family protein
At2g30540	-1.67	-1.52	-0.32			-1.17	glutaredoxin family protein
At1g15165	-1.21	-0.78	-1.04		-1.64	-1.17	U-box domain-containing protein
At2g35780	-0.69	-0.90	-0.80	-2.46	-0.98	-1.17	serine carboxypeptidase S10 family protein
At3g58070	-0.27	-1.30	-1.89		-1.20	-1.16	zinc finger (C2H2 type) family protein
At2g43910	-0.38	-1.18	-1.06	-0.84	-2.37	-1.16	thiol methyltransferase
At2g37530	-0.78	-1.54	-2.45		0.12	-1.16	expressed protein
At1g64255	-1.06	-1.35	-1.07			-1.16	SWIM zinc finger family protein
At5g66260	0.09	-0.83	-0.36	-2.62	-2.08	-1.16	auxin-responsive protein
At1g70310	-1.70	-1.85	0.07			-1.16	spermidine synthase 2 (SPDSYN2)
At3g27830	-1.38	-0.51	-0.71	-1.34	-1.86	-1.16	50S ribosomal protein L12-1
At1g36920		-0.86	-0.33	-1.59	-1.86	-1.16	hypothetical protein
At1g32660	-0.67	-0.90	-3.08	-0.72	-0.43	-1.16	F-box family protein
At4g13820	-1.01	-1.55	-0.71	-1.36		-1.16	disease resistance family protein
At2g03260	-0.46	-1.31	-2.62	-0.75	-0.65	-1.16	EXS family protein
At3g42628	-1.41	-0.24	0.09	-1.60	-2.64	-1.16	phosphoenolpyruvate carboxylase-related
At3g15480	-0.06	-0.50	-1.13	-2.87	-1.23	-1.16	expressed protein
At5g35970	-0.49	-1.32	-1.62	-1.19	-1.16	-1.16	DNA-binding protein
At1g14280	-1.25	-0.69	-0.50	-1.61	-1.73	-1.16	phytochrome kinase
At3g46550	-0.85	-0.55		-2.07		-1.16	fasciclin-like arabinogalactan family protein
At5g28030	-0.89	-0.86	-2.72		-0.15	-1.16	cysteine synthase
At2g28230	-0.86	-1.06	-1.40	-0.88	-1.58	-1.15	expressed protein
At1g49570	-1.04	-1.53	-0.89			-1.15	peroxidase

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At1g26150	-1.54	-1.05	-0.56	-1.61	-1.01	-1.15	protein kinase family protein
At3g04840	-0.64	-1.02	-1.69	-1.20	-1.20	-1.15	40S ribosomal protein S3A
At1g04040	-0.93	-0.26	-0.18	-2.36	-2.03	-1.15	acid phosphatase class B family protein
At2g30390	-1.19	-0.44	-0.29	-1.71	-2.13	-1.15	ferrochelatase II
At1g80060	-0.35	-0.36	-1.84	-1.48	-1.73	-1.15	expressed protein
At3g51470	-1.34	-0.57	-0.51	-1.44	-1.87	-1.15	protein phosphatase 2C
At5g37290	-1.06	-0.87	-1.86	-1.10	-0.84	-1.15	armadillo/beta-catenin repeat family protein
At3g19450	-0.95	-0.82	-0.36	-1.67	-1.92	-1.15	cinnamyl-alcohol dehydrogenase
At5g39430	-0.31	-1.54	-1.59			-1.15	hypothetical protein
At1g16400	-1.74	-0.72	0.08		-2.20	-1.15	cytochrome P450 family protein
At5g19600	-0.86	-0.14	-2.63	-0.61	-1.49	-1.15	sulfate transporter
At5g42280	-0.43	-1.75	-0.19	-1.82	-1.54	-1.15	DC1 domain-containing protein
At2g47240	-1.47	-1.48	0.32	-1.69	-1.40	-1.14	long-chain-fatty-acid CoA ligase family protein
At5g46380	-1.04	-1.46	-0.93			-1.14	hypothetical protein
At3g04890	-0.81	-2.13	-0.64	-1.31	-0.83	-1.14	expressed protein
At1g58480	-1.64	-1.28	-1.34	-1.05	-0.41	-1.14	GDSL-motif lipase
At1g08380	-2.00	-0.95	-0.35	-1.85	-0.57	-1.14	expressed protein
At2g25605	-0.92	-0.69	-1.64	-1.29	-1.15	-1.14	expressed protein
At1g20925	-1.08	-0.30	-0.98	-2.19		-1.14	auxin efflux carrier family protein
At5g20720	-0.45	-0.67	-0.12	-2.31	-2.13	-1.14	20 kDa chaperonin
At1g71480	-0.42	-0.62	-0.44	-1.73	-2.47	-1.14	nuclear transport factor 2
At5g02710	-1.01	-0.85	-0.26	-1.84	-1.72	-1.13	expressed protein
At3g25520	-0.6	-0.82	-0.37	-2.10	-1.78	-1.13	60S ribosomal protein
At3g20200	-0.77	-1.54	-2.02		-0.21	-1.13	protein kinase family protein
At5g22800	-1.16	-0.81	-0.25	-1.95	-1.50	-1.13	aminoacyl-tRNA synthetase family protein
At2g21140	-0.61	-1.58	-0.30	-1.99	-1.18	-1.13	hydroxyproline-rich glycoprotein family protein
At5g23740	-0.96	-0.38	-0.25	-2.30	-1.78	-1.13	40S ribosomal protein S11
At5g15050	-1.29	-1.12			-0.99	-1.13	glycosyltransferase family 14 protein
At1g28410	-1.19	-1.20	-1.08	-1.06		-1.13	expressed protein
At1g32610	-0.25	-1.12	-0.24	-1.66	-2.39	-1.13	hydroxyproline-rich glycoprotein family protein
At4g34280	-0.68	-0.58	-2.39		-0.86	-1.13	transducin family protein
At2g20270	-0.14	-0.33	-0.80	-2.61	-1.77	-1.13	glutaredoxin family protein
At5g14910	-1.05	-1.17	-0.34	-1.94	-1.14	-1.13	heavy-metal-associated domain-containing protein
At2g21340	-0.68		-1.30	-1.22	-1.31	-1.13	enhanced disease susceptibility protein
At2g34357	-1.17	-1.43	-1.35	-0.55		-1.12	expressed protein
At1g19080	-0.35	-0.82	-1.29		-2.05	-1.12	expressed protein
At4g11175	-0.19	-0.72	-1.17	-1.30	-2.24	-1.12	translation initiation factor IF-1
At1g70680	-0.70	-1.53	-0.70	-1.25	-1.43	-1.12	calcosin-related family protein
At5g17010	-0.68	-1.15	-0.78	-1.57	-1.43	-1.12	sugar transporter family protein
At2g15620	-0.87	-0.39	-0.24	-1.61	-2.49	-1.12	ferredoxin nitrite reductase
At5g49720	-0.99	-1.23	-1.33	-1.35	-0.70	-1.12	endo-1,4-beta-glucanase
At4g39460	-1.04	-1.28	0.26		-2.42	-1.12	mitochondrial substrate carrier family protein
At3g45050	-0.54	-1.08	-0.57	-2.00	-1.40	-1.12	expressed protein
At5g35740	-0.61	0.02	-0.63	-1.94	-2.42	-1.12	glycosyl hydrolase family protein 17
At2g47820	-1.40	-1.74	-2.04	-0.48	0.07	-1.12	expressed protein
At2g21480	-1.34	-1.53	-2.17	-0.44	-0.10	-1.12	protein kinase family protein
At4g37040	-0.17	-0.57	-1.88	-1.29	-1.67	-1.12	metallopeptidase M24 family protein
At1g53520	-1.96	-0.95	-0.44			-1.12	chalcone-flavanone isomerase-related
At5g16580	-1.66	-1.31	-0.37			-1.11	glycosyl hydrolase family 1 protein
At5g04050	-0.72	-1.51	-2.19		-0.04	-1.11	maturase-related
At3g56960		-0.77	-0.44	-2.13		-1.11	phosphatidylinositol-4-phosphate 5-kinase
At1g65390	-0.41	-0.87	-1.08	-2.12	-1.10	-1.11	disease resistance protein
At4g22790	-1.78	-1.20	-2.55	-0.30	0.27	-1.11	MATE efflux family protein
At5g03080	-1.12	-1.19	-1.10	-0.57	-1.57	-1.11	phosphatidic acid phosphatase-related
At4g39620	-1.09	-1.17	0.73		-2.91	-1.11	pentatricopeptide (PPR) repeat-containing protein
At4g10120	-0.50	-0.63	-0.86	-1.54	-2.01	-1.11	sucrose-phosphate synthase
At3g22150	-1.50	-0.37	-0.47	-2.16	-1.04	-1.11	pentatricopeptide (PPR) repeat-containing protein
At1g47660	-0.41	-0.35	-0.50		-3.16	-1.11	hypothetical protein
At1g73990	-0.22	-0.39	-1.42	-1.73	-1.77	-1.11	peptidase U7 family protein
At5g21060	-0.73	-0.68	-2.28	-1.15	-0.69	-1.11	homoserine dehydrogenase family protein
At4g31780	-0.76	-1.30	-1.11	-1.14	-1.22	-1.10	monogalactosyldiacylglycerol synthase
At1g67840	-0.67	-0.71	-1.51	-1.21	-1.42	-1.10	ATPase-like domain-containing protein
At1g15930	-0.67	-0.57	-1.91	-1.44	-0.93	-1.10	40S ribosomal protein S12
At3g07110	-1.07		-0.26	-1.76	-1.33	-1.10	60S ribosomal protein L13A
At4g33680	-1.16	-0.36	0.04	-2.57	-1.47	-1.10	aminotransferase class I and II family protein
At1g54020	0.02	-0.22	-2.17		-2.04	-1.10	myrosinase-associated protein
At5g33280	-0.84	-0.78	-2.30	-0.93	-0.66	-1.10	chloride channel-like (CLC) protein
At1g52830	-0.87	-0.95	-1.48			-1.10	auxin-responsive protein
At2g43900	-0.34	-0.88	-0.91	-1.57	-1.80	-1.10	endonuclease/exonuclease
At1g63130	-1.19	-1.05	-0.87		-1.28	-1.10	pentatricopeptide (PPR) repeat-containing protein
At3g23400	-1.16	-1.05	-0.56	-1.67	-1.07	-1.10	plastid-lipid associated protein PAP
At3g52290	-0.71	-1.53	-0.64	-1.66	-0.96	-1.10	calmodulin-binding family protein
At3g04550	-0.87	-0.96	-0.32	-2.21	-1.14	-1.10	expressed protein

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At1g72640	-1.21	-0.50	-1.09		-1.59	-1.10	expressed protein
At3g49470	-0.80	-0.83	0.25	-2.21	-1.91	-1.10	nascent polypeptide-associated complex
At2g23670	-0.65	-1.38	-0.62	-1.85	-0.98	-1.10	expressed protein
At5g53860	-0.99	-0.77	-0.64	-1.70	-1.39	-1.10	expressed protein
At1g44780	-0.76	-0.86	-2.64	-0.90	-0.34	-1.10	expressed protein
At2g15290	-1.13	-0.62	0.13	-2.31	-1.56	-1.10	expressed protein
At2g35810	-0.67	-1.99	-1.57	-0.75	-0.49	-1.09	expressed protein
At5g55820	-1.02	-1.87	-1.92		0.44	-1.09	expressed protein
At3g54470	-1.25	-1.30	-0.23	-1.53	-1.14	-1.09	uridine 5'-monophosphate synthase
At2g45730	-1.08	-0.65	-1.54			-1.09	eukaryotic initiation factor 3
At5g07860	-0.82		-1.56		-0.89	-1.09	transferase family protein
At4g35905	-1.48	-1.39	-0.50	-1.24	-0.84	-1.09	expressed protein
At5g51200	-0.94	-0.59	-0.27	-2.37	-1.27	-1.09	expressed protein
At1g21140	-1.01	-0.73	-1.52			-1.09	nodulin
At1g20850	-1.00	-0.32	-2.74		-0.29	-1.09	cysteine endopeptidase
At1g76160	-0.25	-1.42	-0.40	-2.02	-1.35	-1.09	multi-copper oxidase type I family protein
At2g45820	-0.62	-0.83	-1.09	-1.67	-1.23	-1.09	DNA-binding protein
At5g38360	-0.86	-0.35	-1.65		-1.49	-1.09	esterase/lipase/thioesterase family protein
At3g08010	-0.41	-1.00	-0.55	-1.39	-2.07	-1.08	expressed protein
At5g35475	-0.71	-0.92	-0.07	-1.63	-2.09	-1.08	hypothetical protein
At2g15470	-1.20	-0.68	-1.36			-1.08	glycoside hydrolase family 28 protein
At1g10095	0.34	-1.42	-0.90		-2.35	-1.08	protein prenyltransferase alpha subunit-related
At1g68080	-0.89	-1.18	-1.13	-1.48	-0.71	-1.08	expressed protein
At5g16390	-1.03	-1.09	-0.56	-1.34	-1.38	-1.08	biotin carboxyl carrier protein 1
At2g26340	-0.06	-1.14	-1.15	-1.45	-1.59	-1.08	expressed protein
At1g15500	-0.75	0.14	-2.24	-1.35	-1.18	-1.08	chloroplast ADP, ATP carrier protein
At1g11820	-1.13	-0.19	-0.44	-2.04	-1.58	-1.08	glycosyl hydrolase family 17 protein
At5g39290	-1.25	-1.45	-0.53			-1.08	expansin (EXP26)
At4g18600	-0.67	-1.09	-1.47			-1.08	expressed protein
At3g16010	-0.54	-0.29	-0.61	-1.97	-1.98	-1.08	pentatricopeptide (PPR) repeat-containing protein
At2g23120	-0.56	-1.35	-1.58	-1.59	-0.29	-1.07	expressed protein
At3g61340	-1.18	-1.08	-0.06	-1.55	-1.50	-1.07	F-box family protein
At2g13810	-0.67		-0.55	-0.94	-2.13	-1.07	aminotransferase class I and II family protein
At3g61870	-0.38	-0.37	-1.28	-1.56	-1.76	-1.07	expressed protein
At3g13040	-1.24	-1.57	-1.05	-0.25	-1.25	-1.07	myb family transcription factor
At3g60320	-1.37	-0.61	-1.47	-1.31	-0.59	-1.07	expressed protein
At5g19710	-0.89	-1.33	-0.99			-1.07	hypothetical protein
At3g60990	-0.80	-2.10	0.22	-0.70	-1.96	-1.07	expressed protein
At2g17160	-0.54	-0.04	-1.44		-2.25	-1.07	protein kinase-related
At2g14780	-0.29	-1.56	-1.36			-1.07	hypothetical protein
At1g51850	-1.71	-0.88	-1.35		-0.33	-1.07	leucine-rich repeat protein kinase
At1g12100	-0.94	-1.68	-1.02	-1.02	-0.68	-1.07	protease inhibitor/seed storage/LTP
At5g55010	-0.20	-1.08	-1.91			-1.07	hypothetical protein
At1g64390	-0.71	-1.25	-0.86	-0.61	-1.89	-1.07	endo-1,4-beta-glucanase
At2g01910	-1.00	-1.99	-0.18	-0.99	-1.16	-1.07	microtubule associated protein
At4g32800	-1.10	-2.17			0.07	-1.06	AP2 domain-containing transcription factor
At1g61310	-1.13	-1.23	-0.83			-1.06	disease resistance protein
At1g08280	-0.52	-0.42	-0.97	-1.3	-2.11	-1.06	glycosyl transferase family 29 protein
At1g06360	-0.94	-0.29	-0.24		-2.8	-1.06	fatty acid desaturase family protein
At3g19690	-1.27	-1.61	-1.41		0.03	-1.06	pathogenesis-related protein
At3g53130	-1.55	-1.33	0.19	-1.54	-1.08	-1.06	cytochrome P450 family protein
At3g18524	-0.73	-0.37	-2.08			-1.06	DNA mismatch repair protein MSH2
At1g01100	-0.43	-0.84	-1.76	-1.16	-1.12	-1.06	60S acidic ribosomal protein P1
At3g47675	-0.48	-0.78	-1.77	-1.28	-1.00	-1.06	expressed protein
At2g37020	-1.06	-0.21			-1.91	-1.06	translin family protein
At1g16590	-0.46	-0.93	-0.80	-1.80	-1.30	-1.06	mitotic spindle checkpoint protein
At4g19500	-0.35	-0.20	-1.53	-2.05	-1.17	-1.06	disease resistance protein
At1g67320	-1.13	-2.17	0.12			-1.06	DNA primase, large subunit family
At1g76110	-0.96	-0.08	-1.24	-1.34	-1.68	-1.06	high mobility group (HMG1/2) family protein
At1g20360		-0.70	-0.18	-2.29		-1.06	F-box protein-related
At1g04270	-0.43	-0.78	-1.41	-1.61	-1.05	-1.06	40S ribosomal protein S15
At3g61690	-0.97	-1.35	-1.37	-0.45	-1.15	-1.06	expressed protein
At2g44050	-1.38	-0.84	-0.67	-1.54	-0.85	-1.06	6,7-dimethyl-8-ribityllumazine synthase
At1g01430	-0.93	-0.68	-1.00	-1.51	-1.16	-1.05	expressed protein
At1g19000	-0.32	-1.23	-1.25	-0.98	-1.49	-1.05	myb family transcription factor
At5g58120	-0.83	-1.17	-0.47	-1.69	-1.10	-1.05	disease resistance protein
At5g22020	-0.99	-1.34	0.18	-2.06		-1.05	strictosidine synthase family protein
At5g15220	0.07	-1.10		-2.12		-1.05	ribosomal protein L27 family protein
At3g06035	-0.64	-0.8	-0.40		-2.36	-1.05	expressed protein
At1g63870	-0.88	-0.73	-1.79	-0.54	-1.31	-1.05	disease resistance protein
At1g37015	-0.64	-0.12	-2.54	-0.75	-1.19	-1.05	hypothetical protein
At5g34460	-1.26	-0.58	-1.30			-1.05	replication protein-related
At4g00100	-0.43	-1.33	-0.63	-1.65	-1.22	-1.05	40S ribosomal protein S13

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At2g43340	-0.64	-0.21	-0.63	-1.83	-1.93	-1.05	expressed protein
At3g10050	-0.05	-1.46	-1.68	-1.10	-0.95	-1.05	threonine ammonia-lyase
At2g33370			-0.13	-1.91	-1.10	-1.05	60S ribosomal protein L23
At2g32970	-0.70	-1.19	-0.89	-0.79	-1.66	-1.05	expressed protein
At4g12520	-1.03	-1.76	-1.19	-1.26		-1.05	protease inhibitor/seed storage/LTP
At1g67410	-0.96	-0.91	-2.34	-0.97	-0.05	-1.05	exostosin family protein
At4g33960	-1.03	-0.33	-1.60	-2.05	-0.22	-1.04	expressed protein
At1g13640	-0.72	-0.36	-0.39	-1.93	-1.82	-1.04	phosphatidylinositol 3- and 4-kinase family protein
At2g46990	-0.79	-1.83	-1.73	-0.65	-0.22	-1.04	auxin-responsive protein
At2g23740	-0.38	-0.20	-0.43	-1.78	-2.43	-1.04	zinc finger (C2H2 type) family protein
At4g00030	-0.44	-0.30		-1.57	-1.88	-1.04	plastid-lipid associated protein PAP
At3g03170		-0.95	-1.60	-1.27	-0.35	-1.04	expressed protein
At5g17400	-1.26	-0.86	-1.01	-1.02	-1.07	-1.04	ADP, ATP carrier protein
At5g16710	-0.31	-0.68	-0.10	-2.55	-1.57	-1.04	dehydroascorbate reductase
At4g31370	-0.76	-1.06	-1.31			-1.04	fasciclin-like arabinogalactan family protein
At1g07770	-0.56	-1.12	-0.66	-1.65	-1.22	-1.04	40S ribosomal protein S15A
At1g70090	-0.25	-1.14	-1.51	-1.50	-0.81	-1.04	glycosyl transferase family 8 protein
At5g09280	-0.86	-1.26	-1.01			-1.04	pectate lyase family protein
At4g11100	-0.62	-1.02	-0.54	-1.77	-1.24	-1.04	expressed protein
At4g11480	-1.20	-1.66	-0.53	-0.87	-0.94	-1.04	protein kinase family protein
At1g47220	-0.69	-1.21	-0.44	-1.27	-1.59	-1.04	cyclin
At4g31140	-0.83	-0.50	0.20		-3.02	-1.04	glycosyl hydrolase family 17 protein
At3g61220	-0.78	-0.31	-0.44	-1.03	-2.62	-1.04	short-chain dehydrogenase
At5g53880	-0.67	-0.96	-1.36	-1.31	-0.87	-1.04	expressed protein
At4g24480	-0.70	-1.16	-1.19	-1.09		-1.03	serine/threonine protein kinase
At2g06140	-0.93	-1.41	-1.63		-0.16	-1.03	hypothetical protein
At2g42540	-1.20	-1.00	-1.54	-1.35	-0.08	-1.03	cold-responsive protein
At5g10810	-0.18	-0.54	-0.72	-1.36	-2.36	-1.03	enhancer of rudimentary protein
At4g18730	-0.79	-0.86	-0.47	-1.62	-1.41	-1.03	60S ribosomal protein L11
At1g67430	-0.45	-1.63	-0.95	-1.16	-0.98	-1.03	60S ribosomal protein L17
At2g13690	-1.51	-1.94	-1.77	0.54	-0.46	-1.03	PRLI-interacting factor
At1g52910	-1.14	-0.87	-1.08			-1.03	expressed protein
At1g32070	-0.95	-0.81	-1.63	-1.01	-0.74	-1.03	GCN5-related N-acetyltransferase
At4g03210	-1.03	-1.41	-0.21	-1.54	-0.95	-1.03	xyloglucan:xyloglucosyl transferase
At4g22220	-0.48	-2.27	-1.40	-0.57	-0.40	-1.03	iron-sulfur cluster assembly complex protein
At4g16990	-0.47	-0.92	-2.09	-1.11	-0.54	-1.02	disease resistance protein
At3g62870	-0.45	-1.15	-1.10	-1.41	-1.01	-1.02	60S ribosomal protein L7A
At4g13220	-0.65	-0.73	0.18	-2.22	-1.71	-1.02	expressed protein
At2g02470	-0.24	-0.63	-2.16		-1.06	-1.02	PHD finger family protein
At1g30850	-0.51	-0.67	-0.53	-2.68	-0.71	-1.02	hypothetical protein
At2g02100	-0.71	-1.10	-2.04	-1.74	0.48	-1.02	plant defensin-fusion protein
At4g37780	-0.60	-0.28	-0.45	-1.8	-1.96	-1.02	myb family transcription factor (MYB87)
At3g51920	-0.63	-1.11	-1.37	-1.94	-0.04	-1.02	calmodulin-9 (CAM9)
At5g55110	-1.26	-0.95	-1.00	-1.10	-0.78	-1.02	stigma-specific Stig1 family protein
At1g31580	-1.22	-1.41	-0.66	-1.28	-0.51	-1.02	expressed protein
At1g58350	-1.00	-1.28	-0.77			-1.02	expressed protein
At3g04920	-0.76	-1.54	-0.33	-1.57	-0.87	-1.02	40S ribosomal protein S24
At4g21600	-0.54	-1.32	-1.19			-1.02	bifunctional nuclease
At1g08200	-0.30	-1.50	-0.75	-1.32	-1.21	-1.01	expressed protein
At2g30170	-0.76	-0.78	0.13	-1.86	-1.80	-1.01	expressed protein
At3g06060	-0.41	-0.69	-0.65	-1.13	-2.19	-1.01	short-chain dehydrogenase
At5g03120	-0.07	-0.43	-2.41	-1.05	-1.10	-1.01	expressed protein
At4g18570	-0.73	-1.44	-0.87			-1.01	proline-rich family protein
At4g15690	-0.71	0.19	-1.89	-1.90	-0.76	-1.01	glutaredoxin family protein
At5g15450	-0.40	-1.05	-1.59	-1.10	-0.92	-1.01	heat shock protein 100
At5g58770	-1.12	-1.33	-1.08	-1.44	-0.09	-1.01	dehydrodolichyl diphosphate synthase
At4g19840	-0.33	-1.56	-1.34	-1.16	-0.67	-1.01	lectin-related similar to PP2 lectin polypeptide
At4g14390	-2.01	-0.95	0.21	-1.95	-0.34	-1.01	ankyrin repeat family protein
At4g12050	-1.53	-1.44	-0.88		-0.20	-1.01	DNA-binding protein-related
At2g16730	-0.53	-0.78	-1.16		-1.57	-1.01	glycosyl hydrolase family 35 protein
At3g06510	-0.46	-0.08	-1.07	-0.97	-2.47	-1.01	glycosyl hydrolase family 1 protein
At3g62980	-0.73	-1.20	-1.59	-0.95	-0.56	-1.01	transport inhibitor response 1
At5g62830	-1.69	-1.06	-1.19	-0.74	-0.36	-1.01	F-box family protein-related
At4g38770	-0.05	-1.14	-0.88	-1.60	-1.36	-1.01	proline-rich family protein (PRP4)
At4g01470	-0.81	-1.15	-2.10	-0.88	-0.09	-1.01	major intrinsic family protein
At1g71120	-1.35	-1.25	-0.41			-1.00	GDSL-motif lipase/hydrolase family protein
At2g13790	-1.03	-0.59	-2.03	-1.23	-0.14	-1.00	leucine-rich repeat family protein
At2g36830	-0.13	-0.83	-0.98	-1.52	-1.56	-1.00	major intrinsic family protein
At4g11950	-0.29	-0.54	-2.18			-1.00	hypothetical protein
At5g64050	-0.24	-0.83	-0.93	-1.36	-1.66	-1.00	glutamate-tRNA ligase family protein
At4g13670	-0.55	-1.02	-0.69	-1.13	-1.62	-1.00	peptidoglycan-binding domain-containing protein
At2g07190	-0.94	-1.74	-0.20	-1.35	-0.78	-1.00	hypothetical protein
At2g27630	-0.43	-0.59	-0.25	-2.43	-1.32	-1.00	ubiquitin carboxyl-terminal hydrolase-related

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Locus	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Description
At1g29840	-1.12	-1.18	-0.34	-1.14	-1.22	-1.00	esterase/lipase/thioesterase family protein
At2g24693	-0.61	-0.52	-1.88			-1.00	expressed protein
At4g31800	-0.77	-1.09	-1.47	-1.00	-0.68	-1.00	WRKY family transcription factor
At2g44230	-0.49	-0.76	-0.86	-1.56	-1.33	-1.00	expressed protein
At1g30080	-2.13	-1.56	0.68			-1.00	glycosyl hydrolase family 17 protein
At3g44940	-0.50	-1.52	-0.97			-1.00	expressed protein
At4g35450	-0.38	-0.79	-0.46	-1.67	-1.69	-1.00	ankyrin repeat family protein
At1g53090	-0.96	-0.67			-2.36	-1.00	WD-40 repeat family protein
At3g09500	-0.31	-0.96	-0.47	-1.89	-1.35	-1.00	60S ribosomal protein L35
At1g79420	-1.75	-1.40	0.16			-1.00	expressed protein
At4g25830	-0.31	-1.06	-2.17	-1.01	-0.43	-1.00	integral membrane family protein
At5g06390	-1.75		0.21	-1.10	-1.35	-1.00	beta-Ig-H3 domain-containing protein

Appendix B

Microarray Spatial Experiment

B.1 *Arabidopsis* genes significantly up-regulated close to the lesion (0-6 mm) after treatment of *Arabidopsis* leaf tissue with *Botrytis cinerea*. The experiment was replicated three times (Rep 1-3) and only genes significantly induced more than 2 fold (log of 1) on average are shown.

Locus	Rep 1	Rep 2	Rep 3	Average	Description
At5g44420	6.26	4.10	7.57	5.97	plant defensin protein (PDF1.2a)
At2g26010	5.94	3.47	7.28	5.56	plant defensin-fusion protein (PDF1.3)
At1g75830	4.31	4.35	7.42	5.36	plant defensin-fusion protein
At3g59930	3.16	4.66	5.66	4.49	expressed protein
At1g02400	3.83	3.92	4.93	4.23	gibberellin 2-oxidase
At2g32190	3.57	3.66	5.34	4.19	expressed protein
At5g33355	3.43	4.28	4.66	4.12	expressed protein
At1g55010	4.19	3.38	4.24	3.93	plant defensin-fusion protein (PDF1.5)
At4g11650	3.72	2.72	5.28	3.91	osmotin-like protein (OSM34)
At3g01420	3.39	3.34	4.99	3.91	pathogen-responsive alpha-dioxygenase
At1g73260	2.47	3.50	5.55	3.84	trypsin and protease inhibitor
At5g20230	2.54	3.48	5.27	3.76	Plastocyanin-like domain-containing protein
At5g44430	3.21	1.85	6.15	3.74	plant defensin-fusion protein (PDF1.2c)
At2g29350	3.56	3.79	3.85	3.73	tropinone reductase
At2g26020	4.88	2.28	3.95	3.70	plant defensin-fusion protein (PDF1.2b)
At4g16260	3.55	2.99	4.27	3.60	Beta-1,3-glucanase 1
At5g64905	2.61	3.52	4.48	3.53	expressed protein
At2g23170	3.26	2.66	4.63	3.52	auxin-responsive
At2g36295		2.60	4.41	3.51	expressed protein
At1g33730	2.19	2.75	5.34	3.43	cytochrome P450
At5g54510	3.59	3.43	3.24	3.42	auxin-responsive
At2g31945	2.92	2.85	4.30	3.36	expressed protein
At5g04960	2.08	2.64	5.21	3.31	pectinesterase family protein
At2g18680	3.13	3.91	2.89	3.31	expressed protein
At1g02390	2.50	2.15	5.25	3.30	phospholipid/glycerol acyltransferase
At1g54570	2.37	3.54	3.93	3.28	esterase/lipase/thioesterase
At3g22600	2.87	3.05	3.86	3.26	protease inhibitor/seed storage/LTP
At5g43580	1.44	2.95	5.27	3.22	protease inhibitor
At1g36640	1.45	2.73	5.43	3.20	expressed protein
At1g26400	1.46	3.14	4.93	3.18	FAD-binding domain-containing protein
At2g25450	2.70	3.29	3.54	3.18	2-oxoglutarate-dependent dioxygenase
At2g29170	2.92	2.16	4.42	3.17	short-chain dehydrogenase/reductase
At2g33570	3.31	2.79	3.38	3.16	expressed protein
At4g19305		4.07	2.25	3.16	hypothetical protein
At2g29150	2.57	3.76	3.10	3.15	tropinone reductase
At5g11520	1.95	3.03	4.42	3.13	aspartate aminotransferase
At2g29360	2.03	3.49	3.85	3.12	tropinone reductase
At5g07990	2.59	3.19	3.51	3.10	flavonoid 3'-monooxygenase
At1g14870	2.55	2.71	4.00	3.09	expressed protein
At1g02660	1.51	2.60	5.14	3.08	lipase class 3
At2g15220	2.96	1.97	4.15	3.03	secretory protein
At1g17420	2.19	2.27	4.63	3.03	lipoxygenase
At1g63410		3.24	2.82	3.03	expressed protein
At5g48180	2.48	2.90	3.69	3.03	kelch repeat-containing protein
At1g03220	2.38	3.04	3.63	3.02	Extracellular dermal glycoprotein
At1g30135	2.32	2.01	4.68	3.00	expressed protein
At1g12910	3.47	2.26	3.13	2.96	flower pigmentation protein
At3g55970	1.79	2.64	4.40	2.94	oxidoreductase, 2OG-Fe(II) oxygenase
At1g06520	1.49	3.08	4.24	2.94	phospholipid/glycerol acyltransferase
At5g35525	2.82	3.09	2.86	2.92	expressed protein
At5g48610	1.79	2.82	4.10	2.90	expressed protein
At3g53600	2.21	2.64	3.83	2.89	Zinc finger (C2H2 type) protein
At1g26380	2.52	2.63	3.44	2.86	FAD-binding domain-containing protein
At4g15610	2.74	1.90	3.92	2.85	integral membrane family protein
At3g14770	2.79	2.43	3.29	2.84	nodulin MtN3 family protein
At3g22370	2.22	3.02	3.23	2.82	alternative oxidase 1a
At2g22880	1.74	2.96	3.73	2.81	VQ motif-containing protein
At5g39520	2.96	2.34	3.08	2.79	expressed protein
At2g35980	1.87	2.41	4.06	2.78	harpin-induced family protein
At4g24380	1.88	1.77	4.68	2.77	expressed protein
At1g30700	3.29	3.59	1.41	2.76	FAD-binding domain-containing protein
At1g15520	2.96	2.03	3.28	2.76	ABC transporter family protein
At1g26420	2.25	2.79	3.24	2.76	FAD-binding domain-containing protein
At2g22470	2.80	2.80	2.62	2.74	arabinogalactan-protein
At5g07440	2.31	2.54	3.36	2.74	glutamate dehydrogenase 2
At2g35730	2.56	2.77	2.83	2.72	heavy-metal-associated
At1g74010	2.23	2.61	3.28	2.70	strictosidine synthase family protein
At1g07590	2.28	2.74	3.02	2.68	pentatricopeptide (PPR) repeat-containing protein
At5g03820	1.97	2.23	3.84	2.68	GDSL-motif lipase/hydrolase
At3g15400	3.37	2.55	2.11	2.68	anther development protein
At1g19180	1.66	2.25	4.09	2.66	expressed protein
At2g26560	2.55	1.69	3.74	2.66	patatin
At2g29480	3.33	2.47	2.16	2.66	glutathione S-transferase

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Locus	Rep 1	Rep 2	Rep 3	Average	Description
At5g45890	2.37	0.95	4.64	2.65	senescence-specific SAG12 protein
At3g15356	2.00	2.28	3.67	2.65	legume lectin family protein
At1g65240	3.24	2.00	2.72	2.65	aspartyl protease
At4g24000	3.18	1.56	3.20	2.65	cellulose synthase
At5g36925	2.47	2.70	2.77	2.64	expressed protein
At3g46680	2.06	2.59	3.27	2.64	UDP-glucosyl transferase family protein
At2g17500	1.77	3.20	2.95	2.64	auxin efflux carrier
At2g45220	2.16	2.09	3.51	2.59	pectinesterase family protein
At5g38710	3.86	1.84	2.03	2.58	proline oxidase
At2g31290	3.03	3.29	1.40	2.57	expressed protein
At3g51660	2.63	2.36	2.73	2.57	MIF family protein
At2g33380	2.30	2.97	2.38	2.55	calcium-binding RD20 protein (RD20)
At5g49480	1.86	2.98	2.78	2.54	sodium-inducible calcium-binding protein (ACP1)
At1g09500	1.68	2.12	3.76	2.52	cinnamyl-alcohol dehydrogenase
At5g55410	3.93	1.64	1.98	2.52	protease inhibitor/seed storage/LTP
At3g52400	2.27	2.37	2.91	2.52	syntaxin (SYP122)
At4g21460	2.51	1.06	3.92	2.50	expressed protein
At5g65300	3.65	2.17	1.64	2.49	expressed protein
At3g28340	2.79	2.74	1.92	2.48	galactinol synthase
At1g62380	3.23	1.82	2.38	2.48	1-aminocyclopropane-1-carboxylate oxidase
At4g37710	2.04	2.88	2.46	2.46	VQ motif-containing protein
At1g57630	2.44	2.30	2.64	2.46	disease resistance protein
At3g18250	2.83	1.70	2.83	2.45	expressed protein
At1g13520	2.73	2.05	2.56	2.45	expressed protein
At2g25735	2.29	2.51	2.51	2.43	expressed protein
At1g08920	1.64	1.67	3.99	2.43	sugar transporter
At5g54170	1.29	2.13	3.88	2.43	expressed protein
At4g05633	1.70	2.43	3.15	2.43	hypothetical protein
At2g02990	2.13	3.80	1.34	2.42	ribonuclease 1 (RNS1)
At3g25760	1.82	3.04	2.40	2.42	early-responsive to dehydration stress protein
At2g32150	2.70	2.04	2.51	2.41	haloacid dehalogenase-like hydrolase
At5g35735	2.10	2.72	2.41	2.41	auxin-responsive
At1g12880	-0.10	3.74	3.60	2.41	MutT/nudix family protein
At2g34600	2.13	2.07	3.00	2.40	expressed protein
At4g39060	2.41	3.00	1.76	2.39	F-box family protein
At3g57990	2.21	2.73	2.19	2.38	expressed protein
At5g27760	1.80	2.48	2.81	2.36	hypoxia-responsive
At1g69930	1.79	2.58	2.71	2.36	glutathione S-transferase
At2g18690	2.55	2.26	2.25	2.35	expressed protein
At1g62300	1.69	1.89	3.46	2.35	WRKY family transcription factor
At1g10585	2.71	1.95	2.37	2.34	expressed protein
At1g61890	2.00	1.81	3.22	2.34	MATE efflux family protein
At2g28430	2.06	2.20	2.77	2.34	expressed protein
At5g67080	2.71	1.71	2.60	2.34	protein kinase family protein
At1g74020	1.53	2.01	3.48	2.34	strictosidine synthase
At1g07610	2.10	2.67	2.25	2.34	metallothionein-like protein 1C (MT-1C)
At5g16010	0.90	3.25	2.85	2.33	3-oxo-5-alpha-steroid 4-dehydrogenase
At3g46840		2.66	1.97	2.32	subtilase family protein
At1g57990	2.07	2.02	2.85	2.32	purine permease-related
At4g37430	2.26	2.58	2.09	2.31	cytochrome P450 81F1
At2g31570	1.70	3.04	2.18	2.31	glutathione peroxidase
At4g25170	1.48	1.35	4.07	2.30	expressed protein
At3g06070	3.48	2.89	0.51	2.29	expressed protein
At3g49120	2.51	2.54	1.82	2.29	peroxidase
At5g13080	2.01	1.97	2.89	2.29	WRKY family transcription factor
At1g02530		2.60	1.97	2.28	multidrug resistance P-glycoprotein
At5g40690	1.45	1.57	3.82	2.28	expressed protein
At1g52100	1.72	2.62	2.45	2.26	jacalin lectin family protein
At3g17810	2.57	2.31	1.90	2.26	dihydroorotate dehydrogenase
At5g26940	1.54	2.53	2.69	2.25	exonuclease family protein
At4g37150	2.27	2.27	2.22	2.25	esterase
At5g67190	1.77	2.90	2.07	2.25	AP2 domain-containing transcription facto
At5g16360	2.22	2.84	1.67	2.24	NC domain-containing protein
At4g33150	1.77	2.47	2.46	2.23	lysine-ketoglutarate reductase
At5g55270	2.48	1.94	2.28	2.23	hypothetical protein
At1g07600	1.04	2.48	3.17	2.23	metallothionein-like protein 1A
At1g25155	1.66	2.17	2.85	2.23	anthranilate synthase beta subunit
At3g23920	0.85	2.04	3.79	2.23	beta-amylase
At2g18650	1.76	2.46	2.43	2.22	zinc finger (C3HC4-type RING finger) protein
At4g39670	2.14	2.17	2.30	2.20	expressed protein
At5g26340	2.67	3.12	0.82	2.20	hexose transporter
At4g23190	1.85		2.55	2.20	protein kinase family protein
At5g56870	2.56	2.95	1.06	2.19	beta-galactosidase

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Locus	Rep 1	Rep 2	Rep 3	Average	Description
At1g50080		1.88	2.49	2.18	hypothetical protein
At2g29440	1.60	2.55	2.40	2.18	Glutathione S-transferase
At4g00238	2.15	2.49	1.89	2.18	DNA-binding storekeeper protein-related
At5g23700	1.86	2.29	2.38	2.18	hypothetical protein
At5g53290	1.74	2.30	2.49	2.18	AP2 domain-containing transcription factor
At3g45500	2.50	2.70	1.32	2.17	hypothetical protein
At5g62150	2.03	2.12	2.35	2.17	peptidoglycan-binding LysM domain-containing protein
At5g58100	1.41	2.39	2.67	2.16	expressed protein
At1g07530	1.37	4.60	0.49	2.15	scarecrow-like transcription factor 14
At5g57510	2.57	2.09	1.80	2.15	hypothetical protein
At1g02920	1.93	2.07	2.46	2.15	glutathione S-transferase
At4g14365	2.38	2.06	2.01	2.15	zinc finger (C3HC4-type RING finger) protein
At3g18910		3.00	1.29	2.15	F-box family protein
At3g23630	2.09	2.10	2.24	2.14	adenylate isopentenyltransferase 7
At3g56690		3.05	1.23	2.14	calmodulin-binding protein
At3g46660	1.05	1.86	3.50	2.14	UDP-glucosyl transferase
At1g54130	2.14	2.14	2.12	2.13	RelA/SpoT protein
At1g78380	1.61	2.26	2.53	2.13	Glutathione S-transferase
At3g11170	0.87	2.37	3.15	2.13	omega-3 fatty acid desaturase
At3g47770	2.12	2.89	1.38	2.13	ABC transporter family protein
At4g01360	1.42	2.23	2.73	2.13	expressed protein
At5g20670	1.46	1.97	2.94	2.12	expressed protein
At4g28085	1.44	2.12	2.80	2.12	expressed protein
At1g53890	2.17	2.80	1.38	2.12	expressed protein
At2g39420	1.67	1.94	2.73	2.11	esterase/lipase/thioesterase
At5g63790	2.29	1.72	2.31	2.11	no apical meristem (NAM) family protein
At5g24280	2.18	2.51	1.64	2.11	expressed protein
At1g59870	1.76	2.08	2.43	2.09	ABC transporter family protein
At5g13190	1.65	1.50	3.11	2.09	expressed protein
At5g38900	2.15	1.12	2.98	2.08	DSBA oxidoreductase
At4g17615	1.72	2.01	2.52	2.08	calcineurin B-like protein 1
At1g11310	2.32	1.67	2.25	2.08	seven transmembrane MLO family protein
At2g47600	1.31	2.02	2.89	2.07	magnesium/proton exchanger
At5g05600	1.47	1.42	3.32	2.07	oxidoreductase, 2OG-Fe(II) oxygenase
At1g61360	2.19	1.74	2.26	2.06	S-locus lectin protein kinase
At4g27410	0.91	2.09	3.19	2.06	no apical meristem (NAM) family protein
At1g79680	2.72	1.29	2.17	2.06	Wall-associated kinase
At1g18020	1.22	2.47	2.47	2.06	12-oxophytodienoate reductase
At1g73750	1.84		2.25	2.05	expressed protein
At3g08720	1.95	1.16	3.01	2.04	serine/threonine protein kinase
At3g50120	1.84	2.27	1.99	2.03	expressed protein
At4g36010	1.82	1.86	2.42	2.03	pathogenesis-related thaumatin family protein
At2g30840	2.68	2.64	0.77	2.03	2-oxoglutarate-dependent dioxygenase
At5g16980	2.07	3.31	0.71	2.03	NADP-dependent oxidoreductase
At5g64310	1.50	2.12	2.46	2.03	arabinogalactan-protein (AGP1)
At3g51840	1.84	2.03	2.19	2.02	short-chain acyl-CoA oxidase
At2g43510	2.11	1.37	2.57	2.02	trypsin inhibitor
At1g52890	2.33	1.26	2.46	2.02	no apical meristem (NAM) family protein
At1g17020	1.51	1.13	3.41	2.01	oxidoreductase, 2OG-Fe(II) oxygenase
At1g10190	3.11	1.42	1.51	2.01	expressed protein
At1g52200	1.84	1.83	2.36	2.01	expressed protein
At1g64480	1.62	2.62	1.78	2.01	calcineurin B-like protein 8
At5g25590	3.10	1.24	1.67	2.01	expressed protein
At5g59570	1.96	0.90	3.16	2.00	myb family transcription factor
At1g50230	2.80		1.21	2.00	protein kinase family protein
At5g54050	1.93	1.84	2.23	2.00	DC1 domain-containing protein
At1g08980	1.55	1.76	2.69	2.00	amidase family protein
At5g08790	1.58	2.27	2.12	1.99	no apical meristem (NAM) family protein
At1g70800	1.46	1.95	2.55	1.99	C2 domain-containing protein
At1g51170	1.95	2.05	1.93	1.98	protein kinase family protein
At1g66760	1.83	1.91	2.19	1.98	MATE efflux family protein
At1g03905	2.12	2.33	1.48	1.97	ABC transporter family protein
At4g17500	1.98	1.56	2.37	1.97	ethylene-responsive element-binding protein 1
At1g45130		2.40	1.54	1.97	beta-galactosidase
At4g09160	1.33	1.99	2.58	1.97	SEC14 cytosolic factor family protein
At4g34150	1.26	1.61	3.03	1.97	C2 domain-containing protein
At5g54140	2.31	2.77	0.81	1.96	IAA-amino acid hydrolase
At5g28237	1.85	1.35	2.69	1.96	tryptophan synthase, beta subunit
At5g53970	1.35	2.11	2.43	1.96	aminotransferase
At1g77740	1.60	2.64	1.64	1.96	1-phosphatidylinositol-4-phosphate 5-kinase
At4g06603	2.18	2.14	1.56	1.96	expressed protein
At3g16090	1.46	1.91	2.51	1.96	zinc finger (C2H2 type) protein (ZAT7)
At3g55980	1.88	2.22	1.77	1.95	zinc finger (CCCH-type) protein

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Locus	Rep 1	Rep 2	Rep 3	Average	Description
At3g48580	1.47	1.59	2.80	1.95	Xyloglucan:xyloglucosyl transferase
At4g15400	-0.10	3.11	2.84	1.95	transferase family protein
At5g61210	2.71	1.95	1.19	1.95	SNAP25 homologous protein
At1g76350	1.18	1.96	2.70	1.95	RWP-RK domain-containing protein
At2g29470	2.57	2.04	1.23	1.95	glutathione S-transferase
At5g07880	2.19	2.29	1.35	1.95	SNAP25 homologous protein
At4g28040	1.81	2.04	1.98	1.94	nodulin MtN21 family protein
At3g17440	2.47	2.46	0.91	1.94	novel plant SNARE 13 (NPSN13)
At5g57890	2.03	1.90	1.90	1.94	anthranilate synthase beta subunit
At3g29034	1.82	1.80	2.21	1.94	expressed protein
At1g68630	1.33	1.84	2.65	1.94	expressed protein
At1g26870	2.63	0.99	2.19	1.93	no apical meristem (NAM) family protein
At5g63900	2.14	2.90	0.76	1.93	PHD finger family protein
At1g09080		1.51	2.35	1.93	luminal binding protein 3
At4g34135	1.90	2.24	1.65	1.93	UDP-glucosyl transferase family protein
At1g18200	1.32	1.78	2.67	1.93	Ras-related GTP-binding
At3g22500	2.39	2.12	1.25	1.92	late embryogenesis abundant protein
At4g30530	1.47	1.39	2.90	1.92	defense-related protein
At5g65380	1.53	1.89	2.33	1.92	ripening-responsive protein
At5g11740	1.33	1.81	2.60	1.92	arabinogalactan-protein (AGP15)
At3g02875	1.88	2.13	1.74	1.91	IAA-amino acid hydrolase 1
At5g18450	3.25	1.27	1.22	1.91	AP2 domain-containing transcription factor
At1g25220	1.97	0.63	3.14	1.91	anthranilate synthase beta subunit
At3g50910	1.49	1.07	3.17	1.91	expressed protein
At1g47128	1.46	1.47	2.77	1.90	cysteine proteinase
At2g40670	2.05	1.24	2.40	1.90	two-component responsive regulator
At4g20200	1.81	2.69	1.19	1.90	terpene synthase
At4g37340	1.38	2.86	1.45	1.90	cytochrome P450 family protein
At1g27420	2.42	1.98	1.29	1.90	kelch repeat-containing F-box family protein
At2g44020	1.66	2.24	1.79	1.90	mitochondrial transcription termination factor-related
At3g27380	1.27	2.64	1.76	1.89	succinate dehydrogenase
At5g65110	1.98	1.53	2.16	1.89	acyl-CoA oxidase
At4g36040	1.42	2.19	2.04	1.88	DNAJ heat shock
At2g37750	1.57		2.19	1.88	expressed protein
At4g21980	1.74	1.90	2.00	1.88	autophagy 8a
At3g26860	1.86	2.88	0.89	1.88	self-incompatibility protein-related
At5g47240	1.60	2.11	1.92	1.87	MutT/nudix family protein
At5g50760	2.53	1.79	1.29	1.87	auxin-responsive
At3g21070	1.69	1.74	2.17	1.87	ATP-NAD kinase family protein
At4g25810	1.40	1.90	2.30	1.87	Xyloglucan:xyloglucosyl transferase
At4g15530	1.36	1.83	2.40	1.86	pyruvate phosphate dikinase
At2g39030	1.78	1.16	2.65	1.86	GCN5-related N-acetyltransferase
At1g08630		2.41	1.32	1.86	L-allo-threonine aldolase-related
At1g74950	1.39	1.17	3.02	1.86	expressed protein
At4g27260	0.73	3.09	1.76	1.86	auxin-responsive
At1g32240	2.08	2.19	1.30	1.86	myb family transcription factor (KAN2)
At4g23990	2.26	1.87	1.44	1.86	cellulose synthase
At5g55750	1.60	1.68	2.29	1.86	hydroxyproline-rich glycoprotein
At1g22410	2.06	1.36	2.14	1.85	2-dehydro-3-deoxyphosphoheptonate aldolase
At3g04720	2.36	0.71	2.46	1.85	hevein-like protein (HEL)
At4g05020	1.44	2.07	2.02	1.85	NADH dehydrogenase-related
At1g32920	1.37	1.59	2.58	1.84	expressed protein
At4g08770	1.74	2.41	1.38	1.84	peroxidase
At5g43760	1.67	2.00	1.86	1.84	beta-ketoacyl-CoA synthase
At3g44870	2.58	2.29	0.66	1.84	S-adenosyl-L-methionine:carboxyl methyltransferase
At5g36920	1.75	2.31	1.46	1.84	expressed protein
At3g04640	1.10	2.17	2.25	1.84	glycine-rich protein predicted proteins
At5g57500		2.21	1.46	1.84	expressed protein
At1g11190	1.60	0.63	3.28	1.84	bifunctional nuclease
At5g61950	2.20	1.92	1.39	1.84	ubiquitin carboxyl-terminal hydrolase-related
At3g19970	1.83	1.03	2.63	1.83	expressed protein
At5g26250	1.37	1.85	2.27	1.83	sugar transporter
At5g35400		2.31	1.35	1.83	tRNA pseudouridine synthase family protein
At3g54640	2.52	1.69	1.26	1.82	tryptophan synthase, alpha subunit
At3g01240	2.73	1.29	1.45	1.82	expressed protein
At1g22570	1.97	1.72	1.77	1.82	POT family protein
At5g44480	3.05	1.20	1.21	1.82	NAD-dependent epimerase/dehydratase
At2g22060	1.51	2.09	1.84	1.81	expressed protein
At4g11390	1.92	1.86	1.60	1.79	DC1 domain-containing protein
At2g14130	1.83	1.62	1.92	1.79	Ulp1 protease family protein
At1g09510	1.56	2.09	1.71	1.79	cinnamyl-alcohol dehydrogenase
At2g48140	1.86	1.96	1.54	1.79	protease inhibitor/seed storage/LTP
At2g02710	1.12	2.00	2.23	1.78	PAC motif-containing protein

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Locus	Rep 1	Rep 2	Rep 3	Average	Description
At3g25770	1.42	2.25	1.65	1.78	allene oxide cyclase
At5g37490	1.96	2.78	0.59	1.78	U-box domain-containing protein
At1g80300	1.63	1.73	1.93	1.76	chloroplast ADP
At5g56980	1.98	1.25	2.06	1.76	expressed protein
At5g60530	1.72	1.84	1.72	1.76	late embryogenesis abundant protein-related
At5g11670	1.22	1.06	2.99	1.76	malate oxidoreductase
At3g58750	1.68	1.95	1.65	1.76	citrate synthase
At5g58750	1.60	2.03	1.64	1.76	wound-responsive protein-related
At1g48070	2.13	1.79	1.33	1.75	expressed protein
At5g54810	3.30	0.94	1.02	1.75	tryptophan synthase, alpha subunit
At1g61560	1.72	1.59	1.94	1.75	seven transmembrane MLO
At5g67630	1.17	2.24	1.84	1.75	DNA helicase
At2g14620	1.76	1.55	1.93	1.75	Xyloglucan:xyloglucosyl transferase
At4g21790	1.22	0.69	3.33	1.74	transmembrane protein-related
At5g19230	1.21	1.35	2.67	1.74	expressed protein
At2g45550	1.49	2.11	1.63	1.74	cytochrome P450 family protein
At2g30960	1.63	1.64	1.95	1.74	expressed protein
At5g34850	0.75	1.04	3.43	1.74	calcineurin-like phosphoesterase
At5g13330	1.90	1.96	1.35	1.74	AP2 domain-containing transcription factor
At3g52240	1.87	1.60	1.72	1.73	expressed protein
At1g02050	1.29	1.48	2.41	1.73	chalcone and stilbene synthase
At5g44360	1.55	3.00	0.63	1.73	FAD-binding domain-containing protein
At5g61820	2.40	1.28	1.51	1.73	expressed protein
At5g53190	2.18	1.53	1.46	1.73	nodulin MtN3 family protein
At1g01470	1.33	1.88	1.97	1.72	late embryogenesis abundant protein
At1g80000	1.31	1.15	2.71	1.72	expressed protein
At3g13650	1.81	1.32	2.02	1.72	disease resistance response protein-related
At1g57980	2.17	2.31	0.66	1.71	purine permease-related
At2g24850	0.69	1.57	2.88	1.71	aminotransferase
At4g37060	2.53	1.39	1.21	1.71	patatin
At5g42650	1.25	1.65	2.23	1.71	allene oxide synthase (AOS)
At5g48540	1.11	1.87	2.14	1.71	33 kDa secretory protein-related
At1g37130	1.45	1.27	2.38	1.70	nitrate reductase 2 (NR2)
At4g23810	2.05	2.20	0.85	1.70	WRKY family transcription factor AR411
At1g05010	0.93	1.68	2.49	1.70	1-aminocyclopropane-1-carboxylate oxidase
At5g33360	2.18	1.91	0.99	1.70	hypothetical protein
At5g56940	1.23	2.15	1.71	1.69	ribosomal protein S16 family protein
At4g00070	1.38	1.21	2.48	1.69	Zinc finger protein-related
At1g05560	2.11	2.19	0.76	1.69	UDP-glucose transferase
At5g01540	1.22	1.87	1.97	1.68	lectin protein kinase
At2g23150	1.37	1.82	1.86	1.68	NRAMP metal ion transporter 3 (NRAMP3)
At1g61820	1.83	1.44	1.76	1.68	glycosyl hydrolase (BGLU46)
At1g61120	2.27	1.26	1.49	1.68	terpene synthase
At1g32450	1.77	0.33	2.92	1.67	POT family protein
At5g61810	1.14	2.09	1.79	1.67	mitochondrial substrate carrier family protein
At1g24909	1.69	0.81	2.52	1.67	anthranilate synthase beta subunit
At3g02150	1.18	1.58	2.25	1.67	TCP family transcription factor
At1g65690	2.17	1.14	1.71	1.67	harpin-induced protein-related
At5g27120	1.58	1.50	1.93	1.67	Zinc finger (C3HC4-type RING finger) protein
At2g43530	1.23	1.61	2.16	1.67	trypsin inhibitor
At5g28050	1.03	1.11	2.87	1.67	cytidine/deoxycytidylate deaminase
At1g28190	1.40	1.42	2.19	1.67	expressed protein
At4g05100	1.97	1.65	1.38	1.67	myb family transcription factor (MYB74)
At5g14560	2.08		1.24	1.66	expressed protein
At5g58170	1.51	1.99	1.48	1.66	glycerophosphoryl diester phosphodiesterase
At4g02140	2.00	2.08	0.88	1.65	expressed protein
At1g03970	1.38	1.85	1.74	1.65	G-box binding factor 4 (GBF4)
At3g43682	1.77	1.51	1.67	1.65	hypothetical protein
At1g18860	-0.03	2.32	2.66	1.65	WRKY family transcription factor
At1g13210	1.52	1.84	1.59	1.65	haloacid dehalogenase-like
At5g65470	1.73	1.69	1.52	1.65	expressed protein
At5g24430	1.46	1.84	1.63	1.64	calcium-dependent protein kinase
At3g06810	1.78	1.56	1.58	1.64	acyl-CoA dehydrogenase-related
At1g30780	2.36	1.27	1.28	1.64	F-box family protein
At1g80100	1.51	1.83	1.58	1.64	phosphotransfer family protein
At5g60510	2.49	1.63	0.79	1.64	undecaprenyl pyrophosphate synthetase
At3g13950	1.22	2.07	1.62	1.64	expressed protein
At2g21120	1.48	1.87	1.56	1.63	expressed protein
At2g14290	1.37		1.89	1.63	F-box family protein
At3g25780	0.92	1.69	2.28	1.63	allene oxide cyclase
At3g06500	0.83	1.61	2.45	1.63	beta-fructofuranosidase
At2g32660	0.16	2.49	2.23	1.63	disease resistance family protein
At2g03170	1.20	1.47	2.21	1.63	E3 ubiquitin ligase

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